Phytochemical screening, bioactive compound characterization and antimicrobial activity assay of *Commelina benghalensis* L. leaf

Ayantu Hinsermu Dinsa

A Thesis Submitted to the Department of Applied Biology

College of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master‘s in Applied Biology (Biotechnology)

Office of Graduate Students

Adama Science and Technology University

November, 2025

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled "**Phytochemical Screening, bioactive compound characterization and antimicrobial assay of *Commelina benghalensis* L. leaf**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Name of student	**Signature**	**Date**

RECOMMENDATION

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Phytochemical screening, bioactive compound characterization and antimicrobial assay of *Commelina benghalensis* L. leaf**" prepared under our guidance by Ayantu Hinsermu submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Biology (Biotechnology). Therefore, we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	**Signature**	**Date**
_____	_____	_____
Co-advisor	**Signature**	**Date**

APPROVAL PAGE

We, the advisors of the thesis entitled "**Phytochemical Screening, bioactive compound characterization and antimicrobial assay of *Commelina benghalensis* L leaf**" and developed by Ayantu Hinsermu; hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	**Signature**	**Date**
Co-advisor	**Signature**	**Date**

APPROVAL OF BOARD OF REVIEWERS

We, the undersigned, members of the Board of Examiners of the thesis by Ayantu Hinsermu have read and evaluated the thesis entitled "**Phytochemical Screening, bioactive compound characterization and antimicrobial assay of *Commelina benghalensis* L. leaf**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Biology (Biotechnology).

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LIST OF ACRONYMS/ABBREVIATIONS

ANOVA	Analysis of Variance
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EOs	Essential Oils
GC	Gas Chromatography
IC50	Inhibition concentration at 50 %
HPLC	High Performance Liquid Chromatography
MBC	Minimum bactericidal concentration
MDR	Multiple drug resistance
MFC	Minimum fungicidal concentration
MIC	Minimum inhibition concentration
MS	Mass spectrum
NMR	Nuclear Magnetic Resonance
Rf	Retention factor
RSA	Radical Scavenging Activity
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TLC	Thin Layer Chromatography
UV	Ultraviolet
WHO	World Health Organization
ZOI	Zone of inhibition

ABSTRACT

C. benghalensis L., traditionally used for treating leprosy, fever, and snake bites. The study aimed to identify bioactive compounds, characterize essential oils, and evaluate the plant's potential as a source of therapeutic agents. Phytochemical screening was conducted on C. benghalensis leaves, followed by compound extraction. The antimicrobial activity of the leaf extract was assessed against selected human pathogens using the disc diffusion method. Antioxidant activity was evaluated using the DPPH assay. MIC, MBC, and MFC were determined using the Broth Dilution Method. The structure of the bioactive compound was determined using GC-MS, NMR and HPL. Data analysis was performed using non-parametric one-way ANOVA and Tukey's test. Methanol yielded the highest extract (23.54 g, 75.27%), followed by chloroform (5.11 g, 16.34%) and petroleum ether (2.62 g, 8.37%), reflecting the solvents' varying abilities to solubilize different phytochemicals. Qualitative phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, steroids, terpenoids, saponins, phenols and coumarins aligned with the known diversity of bioactive compounds in C. benghalensis. GC-MS analysis of the essential oil identified 25 components, with five compounds as the major constituents, highlighting the potential for various applications based on these compounds' properties. Further isolation efforts using TLC and column chromatography led to the identification of 1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester (DEHP), a known plasticizer, confirming its presence in the plant extract. The extracts of C. benghalensis have demonstrated antibacterial activity against various bacterial strains, with inhibition zones ranged from 6±0.6 mm to 15.7±1.5 mm. The essential oil and methanol extracts were found to be the most effective. However, they have shown less effective than standard antibiotics. Furthermore, these extracts also exhibited antifungal activity against C. albicans, with inhibition zones from 8.7±1.2 mm to 16.7±3.1 mm, where the petroleum ether extract showed the highest activity. This comprehensive analysis underscores C. benghalensis's rich phytochemical profile and potential as a source of valuable bioactive compounds, warranting further investigation into its pharmacological and industrial applications.

Keywords: *Antimicrobial, Bioactive compound, C. benghalensis L., Essential oil and*

CHAPTER-1

INTRODUCTION

1.1 Background of the study

Despite significant advancements in modern medicine, microbial diseases persist as substantial global threats. Acknowledging this, the World Health Organization emphasizes herbal medicine as a primary healthcare modality in numerous developing nations. Globally, approximately 80% of the population relies on medicinal plants for disease treatment, with a more pronounced prevalence in African nations (Usure et al., 2024). Ethiopia, recognized for its rich biodiversity and profound traditional knowledge of medicinal plants, significantly incorporates these resources into its healthcare system (Alemu *et al*., 2024).

C. benghalensis is a succulent, astringent troublesome weed native to Africa and Asia. The plant (also called tropical spiderwort) is an exotic, invasive, herbaceous perennial of tropical climate but grows as an annual in temperate regions. The plant which belongs to the family Commelinaceae, possesses 2.5 – 7.5 cm long ovate leaves, erect stem and aerial flowers that are chasmogamous. The leaves of *C. benghalensis* are used traditionally for the treatment of headaches, constipation, snake bites, skin lump and cancer (Okoko, 2021).

Plant extraction is a fundamental process in preparing samples for the evaluation of antimicrobial activity, phytochemical analysis and isolation of pure bioactive molecule. This procedure involves isolating bioactive compounds from plant materials, which can include secondary metabolites such as alkaloids, flavonoids, terpenes, and saponins. Various extraction methods, including maceration, Soxhlet extraction, and percolation, are employed using different solvents tailored to the polarity of the target compounds (Abubakar & Haque, 2020). For instance, polar solvents like methanol and ethanol are effective for extracting hydrophilic compounds, while nonpolar solvents such as hexane are used for lipophilic substances. The extracted compounds are then subjected to phytochemical screening to identify their presence and concentration, followed by antimicrobial testing against specific pathogens to assess their efficacy (Sasidharan *et al*., 2011). This systematic approach not only aids in understanding the therapeutic potential of medicinal plants but also

contributes to the discovery of new antimicrobial agents in the face of rising antibiotic resistance (Kebede *et al.*, 2021).

Essential oil extraction and characterization of pure bioactive compounds is a vital area of research that focuses on isolating aromatic compounds from plant materials for various applications, including pharmaceuticals, cosmetics and food industries. The extraction process employs several techniques, such as hydro-distillation, cold pressing and solvent extraction, each with its unique advantages and suitability for different plant types. These methods enable the recovery of essential oils that contain a complex mixture of volatile and non-volatile compounds, which are responsible for the oils' therapeutic properties (Aziz *et al.*, 2018). Characterizing these bioactive compounds is essential for understanding their chemical composition and potential health benefits, paving the way for their use in developing natural remedies and enhancing product formulations. The ongoing exploration in this field, also contributes to the discovery of new applications in combating various health issues (Sasidharan *et al*., 2011).

The increasing prevalence of antimicrobial resistance poses a significant challenge to global health, necessitating the exploration of alternative therapeutic agents. Medicinal plants have long been recognized for their potential to provide effective antimicrobial compounds (Dubale *et al.*, 2023). This interest is underscored by the historical use of various plants in traditional medicine systems, which have been employed for centuries to treat infectious diseases. Recent studies emphasize the need for systematic evaluations of these plants to validate their efficacy and safety as antimicrobial agents, particularly against multidrug-resistant (MDR) pathogens that are becoming increasingly difficult to treat with conventional antibiotics (Manandhar *et al*., 2019). Researchers utilize a range of methodologies to evaluate the antimicrobial properties of medicinal plants, including phytochemical screening and in vitro antimicrobial assays (Salam *et al*., 2023). Common techniques, such as disk diffusion method and broth micro-dilution, are employed to ascertain the minimum inhibitory concentration (MIC) of plant extracts against specific microorganisms. These approaches not only aid in identifying active compounds but also enhance our understanding of their mechanisms of action. The results from previous studies lend scientific support to the traditional uses of these plants and underscore their potential in the development of new antimicrobial therapies (Prestinaci *et al.*, 2015).

Preliminary studies have shown that extracts from *C. benghalensis* exhibit antimicrobial effects against pathogens such as *C. albicans* and *S. aureus* (Sinha, 2019). However, there is a lack of comprehensive assessments regarding its effectiveness against a wider variety of microorganisms (Cavichi *et al*., 2023). Future investigations should prioritize the standardization of extraction techniques and the identification of specific active compounds that contribute to these antimicrobial properties. There is a pressing need for more in-depth phytochemical studies to isolate and characterize pure bioactive compounds from *C. benghalensis*. Identifying these compounds has the potential to yield novel therapeutic agents; however, existing research has largely concentrated on crude extracts rather than on isolated compounds (Das *et al.*, 2024).

1.2 Statement of the problem

The persistent challenge of infectious diseases continues to pose a significant public health crisis, despite remarkable advancements in biomedical knowledge related to their prevention, treatment, and control. This crisis is primarily driven by the rapid evolution of microbial resistance to existing first-line drugs, which has outstripped our current therapeutic approaches. As a result, there is an urgent need for innovative antimicrobial agents, leading researchers to investigate alternative sources, particularly medicinal plants. These plants not only provide potential therapeutic compounds but also represent a rich reservoir of diverse chemical constituents that can be developed into targeted medications. However, despite the extensive knowledge available about these natural resources, a critical research gap exists in fully understanding and optimizing their pharmacological potential against resistant pathogens. Bridging this gap is essential for developing effective treatment options and improving public health outcomes in the face of rising antimicrobial resistance. In light of that, the present study aimed to investigate the phytochemical composition, characterize a bioactive compound, extract essential oil and evaluate the antimicrobial and antioxidant activities of the extracts derived from the medicinal plant *C. benghalensis* L. leaf.

The traditional use of *C. benghalensis* reflects its significance in ethnomedicine across different cultures. Its diverse applications in treating infertility, skin conditions, digestive issues, and pain highlight the importance of this plant in traditional healing practices. Ongoing research into its phytochemical properties may further validate its therapeutic potential, but its phytochemical and biological properties are not fully understood (Ghosh *et al*., 2019). By conducting phytochemical

screening and antimicrobial and antioxidant testing, the researchers sought to provide scientific evidence on the medicinal potential of the plant species and contribute to the development of new natural product-based therapies.

1.3. Objectives

1.3.1. General objective

To perform phytochemical screening, bioactive compound characterization and evaluation of antimicrobial activity of *C. benghalensis L.* extracts against selected pathogens (Bacterial and Fungal Strains)

1.3.2. Specific objectives

- To assess phytochemical components from leave crude extracts of *C. benghalensis L.*
- To analyze antimicrobial activity of the leaf extracts of *C. benghalensis L*. against selected human pathogens (type strains such as; Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853) and Streptococcus pyogenes (ATCC-19615 and *C. albicans ATCC* 10231)
- To evaluate the antioxidant activity of the extracts
- To explore the structure of the pure bioactive compound

1.4 Scope of the study

The sampling area for this study was from the field of Adama Science and Technology University campus and the study was conducted in Adama Science and Technology University. The study was based on phytochemical qualitative analysis of the presence of various phytochemical classes (e.g., alkaloids, flavonoids, terpenoids, phenols) in the plant extracts. Bioactive compound characterization, identification and structural elucidation of the bioactive compound present in the plant extracts using advanced analytical techniques such as HPLC, NMR, GC-MS. Evaluation of the antibacterial and antifungal properties of the plant extracts against a range of clinically relevant microorganisms (determination of the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the active extracts. The overall scope of the study is to provide a comprehensive understanding of the phytochemical composition, bioactive compounds, antioxidant and antimicrobial potential of *C. benghalensis* L., which can contribute to the development of new natural product-based therapeutic applications.

1.5 Significance of the study

The study on the phytochemical screening, antimicrobial and antioxidant properties of *C. benghalensis* L. leaves is significant because this plant is widely used in traditional medicine, but its therapeutic potential is not fully understood. By investigating the phytochemical composition and bioactive compounds present in the plant, this research can provide scientific evidence to support the rational use of *C. benghalensis* L. in traditional healthcare systems. Additionally, the evaluation of the antimicrobial activities of the plant extracts is particularly important in the context of rising antibiotic resistance. The identification of potential natural antimicrobial agents from this plant could contribute to the development of new, effective, and sustainable treatments. Moreover, the assessment of the antioxidant potential of the plant can shed light on its ability to protect against oxidative stress and associated health benefits, which is valuable for the development of natural product-based therapies and nutraceuticals.

CHAPTER-2

LITERATURE REVIEW

2.1. Medicinal plants

Medicinal plants continue to be vital in the everyday lives of people, especially those living in developing countries across Asia and Africa, including Ethiopia. They not only complement or replace modern medical treatments—often scarce in these regions—but also contribute to the health and well-being of local communities. These plants hold essential roles in daily life and are closely linked to various social, cultural, and economic aspects related to life stages, aging, illness, and death. Used for diagnosing and treating diseases and infections, plants have served as valuable sources of safe and effective medicines since ancient times (Agidew, 2022). The world health organization (WHO) defined traditional medicine as the total combination of knowledge and practices that can be formally explained or used in the prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. About 75–90% of the rural population in the world (excluding western countries) relies on traditional medicines as their only health care system. This is not only because of poverty where people cannot afford to buy expensive modern drugs, but traditional systems are also more culturally acceptable and meet the psychological needs in a way modern medicine does not (Bamola *et al*., 2018)

Most medications used in modern conventional medicine originate from natural sources or are semi-synthetic derivatives of natural products. Screening these natural products is a logical approach to discovering new drugs. Many recently developed antibiotics on the market are derived from natural or semi-synthetic compounds, and approximately 20% of all plant species have undergone biological or pharmaceutical testing. Plants are valuable sources of medicine due to their production of diverse bioactive molecules (Hemalatha *et al*., 2013). The pharmaceutical industries could utilize the bioactive components or plant extracts as a new formulation for the discovery of novel drugs to treat a variety of diseases. Herbal remedies like ashwagandha and brahmi help improve immunity, increase nutrients, repair body cells, and increase energy. In a way that is environmentally sustainable and preserves the biodiversity of these natural resources,

medicinal and aromatic plants can significantly improve the subsistence livelihood of rural people, particularly women (Wolditsadik, 2018).

Furthermore, up to 80% of the world's population currently gets their primary healthcare from traditional medicine, according to the World Health Organization (WHO). As different studies stated that, traditional medicine encompasses all of the knowledge, abilities, and practices that are derived from indigenous theories, beliefs, and experiences of various cultures and are used to prevent, diagnose, treat, or improve physical and mental health conditions. The use of medicinal plants to treat a variety of diseases and the development of indigenous medicines both have significant financial benefits (Gebrehiwet & Gebremichael, 2019).

2.2. Techniques of isolation and purification of bioactive molecules from plants

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity (Technique *et al*., 2013). Many bioactive molecules have been isolated and purified by using paper thin-layer and column chromatographic methods. Column chromatography and thin-layer chromatography (TLC) are still mostly used due to their convenience, economy, and availability in various stationary phases. Silica gel column chromatography and thin-layer chromatography (TLC) have been used for separation of bioactive molecules with some analytical tools. Determination of the structure of certain molecules uses data from a wide range of spectroscopic techniques such as UV-visible, Nuclear Magnetic Resonance (NMR) (Altemimi *et al*., 2017).

2.3 Antimicrobial and antioxidant activities of medicinal plants

2.3.1 Antimicrobial activity of medicinal plants

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious

diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Ríos & Recio, 2005). Ethanolic Leaf extract of *C. benghalensis* showed potential inhibitiory activities against *Streptococcus lactis* (Gram+ve) and *Enterobacter* aerogenes (Gram–ve) bacteria by performing Agar well diffusion method. Phytoconstituents obtained from plant active against plant and human pathogenic bacteria. In Bangladesh, Antimicrobial activities were found on different extracts of plant by disc diffusion method Last few years, multiple drug resistance in both plant and human pathogenic bacteria have been developed due to the random use of commercial antimicrobial drugs which is commonly used in the treatment of infectious diseases (Khanpara, 2019).

2.3.2 Antioxidant activity of medicinal plant

A study investigating the anti-oxidant activity of *C. benghalensis* found a positive correlation between total polyphenols and antioxidant properties of the plant. This plant also provides a great source of dietary antioxidants and possess high antioxidant activities which prevent oxidative damage, slows down the process of aging, reducing the chances of cancer and other cardiovascular and neurological diseases. In another experiment, ethanolic extracts from the leaves of *C. benghalensis* shows to be rich in antioxidants due to the presence of mainly phenolic groups which neutralize free radical. Thus can be successfully used in pharmaceutical industries. Fresh aerial parts of *C. benghalensis* extracted with acetone and methanol shows good antioxidant properties showing the inhibitory ability of free radicals. Reactive oxidative species and oxidative stress increases the chances of diseases like diabetes, cancer heart-diseases, autoimmune diseases, aging and neurological diseases and antioxidative property of this plant prevent such diseases. Presence of flavonoids suggests that this plant have anti-oxidant properties. The free-radical scavenging ability of *C. benghalensis* has been found in a study due to the presence of phenolics (Khanpara, 2019).

2.3.3 Essential oil from medicinal plants

Essential oils are liquid extracts from aromatic plants, which have numerous applications in multiple industries. There are a variety of methods used for the extraction of essential oils such as supercritical fluid extraction, subcritical extraction liquid, solvent-free microwave extraction and conventional (hydrodistillation, steam distillation, hydrodiffusion, solvent extraction), with each method exhibiting certain advantages and determining the biological and physicochemical

properties of the extracted oils. Essential oils from different plant species contain more than 200 constituents which are comprised of volatile and non-volatile components. The application of essential oils as antimicrobial, anticancer, anti-inflammatory and anti-viral agents is due to their effective and efficient properties (Asyikin *et al*., 2018). No paper has reported about essential oil extracted from *C. benghalensis* yet.

2.4. *Commenila benghalensis L.*

2.4.1 Distribution and uses as a traditional medicinal plant

C. benghalensis L. belongs to the family Commelinaceae is a perennial native herb found in most parts of tropical Asia and Africa. This plant is commonly known as *Benghal* dayflower or dew flower. It is a large, meandering herb with roots that have basal nodes that appears once a year and grows up to 40 cm in height. The plant is distinguished by its lovely little bluish-violet flowers. Oval and elliptic leaves are common in plants, but some also have oblong, slightly triangular, dark-bright green, nearly 4-seven-centimeter leaves. The sprees are compressed, green, funnel-shaped, and roughly 1.5 cm long. The plant produces broadly ovoid-oblong capsules that measure 4-5 mm in length. The seeds are void (Tam *et al*., 2016). This pantropical weed grows along roadsides, in waste areas, and in cultivated fields. It is indigenous to both Asia and Africa. It is distributed in Nigeria, Ethiopia, Kenya, Senegal, and Cameroon in Africa (Chioma & Omoregie, 2010).

Taxonomical Classification

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Monocotyledonae
- Order: Commelinales
- Family: Commelinaceae
- Genus: Commelina
- Species: *Commelina benghalensis* (Ghosh et al., 2019).

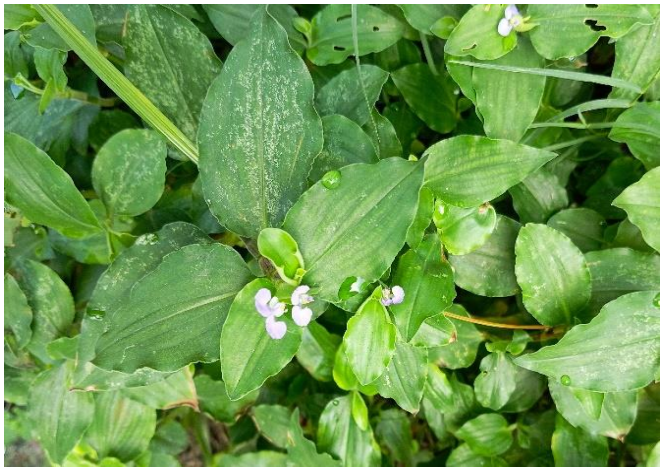


Figure 1*: Commelina benghalensis Linn* (Ghosh *et al.*, 2019)

The literature reveals that *C. benghalensis* is used to treat a number of skin conditions, including eczema, warts, scabies, acne, and sleep disorders, as well as mental illness. It is also used to treat pain, including headaches, toothaches, cataracts, and conjunctivitis (Nazim & Khatun, 2024). The stem extract of C. benghalensis is used as a laxative, diuretic, anti-inflammatory, febrifuge, and for wound healing in Lesotho and Cameroon (Ghosh *et al.*, 2019)The traditional medical system makes use of *C. benghalensis* to treat a variety of illnesses. Pain, constipation, headaches, leprosy, fever, snake bites, and jaundice have all been treated with it. Insanity, psychosis, epilepsy, and mouth thrush have all been treated with it. It is used to treat infertility in women in many tropical Asian countries, and in India, it has anti-inflammatory, depressant, laxative, emollient, bitter, and demulcent properties. *C. benghalensis* is used as a febrifuge and diuretic in China. It is a typical food plant in Africa, Pakistan, and other Asian regions. It is said that this plant has amazing medicinal qualities. This plant's chemical components and extracts have demonstrated significant pharmacological qualities that are used to treat a variety of illnesses (Ghosh *et al*., 2019).

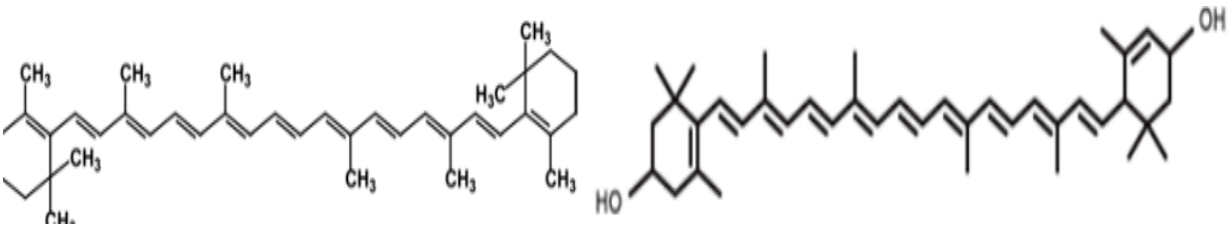
2.4.2 Pharmacological Activities

C. benghalensis, also known as Benghal dayflower which is a perennial medicinal plant inhabitant to tropical Asia and Africa. It shows Pharmacological activities like laxative, anti-inflammatory, anti-microbial, Anti-cancer, sedative, Analgesic, Hepatoprotective, Anti-depressant, Anti-viral, Antioxidant, Antidiarrheal, Demulcent, Emollient, Diuretic and Febrifuge. Overall, many investigations have been done on pharmacological active phytoconstituents of this plant (Khanpara, 2019).

2.4.3. Phytochemicals

Phytochemicals, also known as plant-based bioactive compounds, are naturally synthesized by plants to safeguard themselves. These valuable substances can be obtained from a diverse range of sources, including whole grains, fruits, vegetables, nuts, and herbs. To date, scientists have identified over a thousand different types of phytochemicals (Kumar et al., 2023). Throughout history, phytochemicals have played a vital role in communities around the globe. These plant derived compounds have been utilized in healthcare systems as medicinal remedies for a wide range of ailments. Additionally, phytochemicals serve as valuable starting points for the development of pharmaceutical drugs. Naturally, these bioactive chemicals are synthesized in various parts of the plant, including the bark, leaves, stem, root, flower, fruits, and seeds. It's important to note that the quantity and quality of bioactive chemicals can differ between different plant parts (Samuel & Kumar, 2016).

The extraction of compounds from plant materials forms the foundation of natural product research. The exploration for innovative drugs within medicinal plants entails a process of screening plant extracts to identify new compounds, followed by conducting tests to assess their biological activities. When potential new molecules or bioactive compounds are identified, they are isolated and purified to elucidate their molecular structure and subsequently subjected to further pharmacological or toxicological evaluations. (Bitwell *et al*., 2023). Phytochemical investigations have revealed that the presence of anthocyanins, steroids, iridoids, terpenoids, lignans, dammarane, sterols, hydrocyanic acid and campesterol, flavonoids, aliphatic alcohols, polyols, phenolic acids, volatile waxes, n-octacosanol, n-trioctanol with lutein, and b-carotene found to be present in high quantity in *C. benghalensis* L. (Fibrich & Lall, 2020).



β-carotene Lutein

Figure 2: Lutein and β-carotene isolated from *C. benghalensis* (Tam *et al.*, 2016).

CHAPTER-3

MATERIALS AND METHODS

3.1 Chemicals, culture media, apparatuses and instruments

3.1.1 Chemicals and culture media

The research utilized the following chemicals and reagents: n-hexane (99.9%, Pentokey Organy, India), petroleum ether (Indenta Chemicals, India), ethyl acetate (99.9%, Sisco Research Laboratories, India), chloroform, methanol, and DMSO (99.8%, Loba Chemie, India). Additionally, vanillin (Sisco Research Laboratories, India), ciprofloxacin (Wellona Pharma, India), ascorbic acid, Ketoconazole, Mueller Hinton Agar (Micro express, India), Sabouraud dextrose agar, DPPH (98.5%, China), ethanol, distilled water, and silica gel for column chromatography (60-200 mesh, Merck, India) were acquired from local markets in Addis Ababa, Ethiopia. All solvents and reagents employed were of analytical grade.

3.1.2 Apparatuses and instruments

The apparatus and instruments which have been utilized in current study are: mortar and pestle, electric blender (Shanghai Jingke, JK-HSG-100A, China), polyethylene bag, electronic balance, beakers (different size), shaker (Gemmy Industries, VRN-200, Taiwan), filter paper (Whatman No.1 filter paper, 125 mm diameter, India)), column chromatography set up, separatory funnel, vials, oven, stove, heating mantle, TLC plate (thickness; 0.25 mm, size; 20 x 20 cm Aluminum coated with high-grade silica gel, 230–400 mesh, pore size 60 Å, Merck Grade 64271, Darmstadt, Germany), water bath, UV lamp (UV4AC6/2, Clevenger's apparatus (Aarson Scientific Works, India), flasks (different size), measuring cylinder, rotary evaporator (DW-RE-3000, China), aluminum foil, glass rod, chromatographic chamber, ruler, pencil, spatula, distillation flask, petri dish, refrigerator, holder, incubator, digital melting point (Gebrehiwot et al., 2024). UV-VIS spectrophotometer (Cecil CE4001, UK), GC-MS (Santa Clara, CA, USA) and NMR spectrometers.

3.2. Description of the study area

The fresh leave of *C. benghalensis* L. was collected from Adama Science and Technology University (ASTU) campus. Adama Science and Technology University is located in the capital of East Shewa Zone of Oromia Regional State of Ethiopia, Adama. Adama city is located at about 99 Km south east of Addis Ababa. The geographical coordinate of Adama City is 8.54°N and 39.27°E at an average elevation of 1712 m above sea level. This altitudinal location is locally categorized (baddadaree) (1500-2400m) above sea level. The city is located between the base of an escarpment to the west and the great Rift Valley to the east (File & Dinka, 2020).

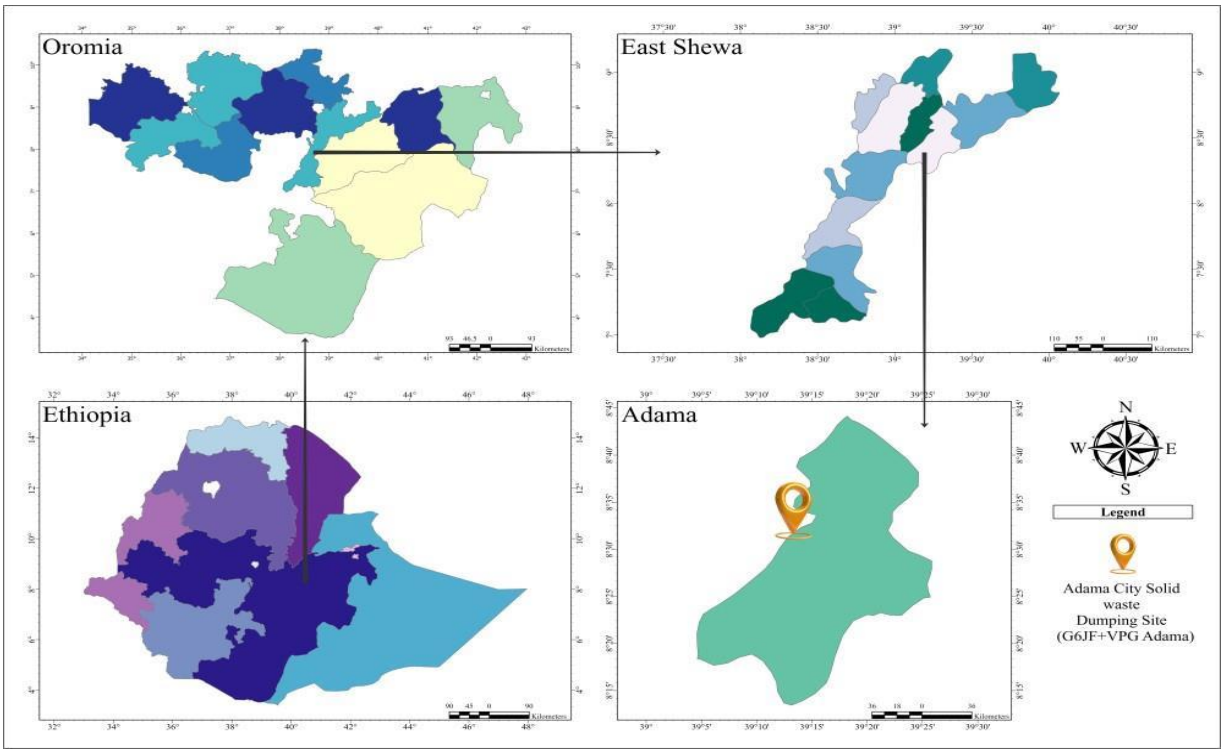


Figure 3: Location of the study area (Around Mini media and Female library)

3.3 Plant material collection and identification

Two kilogram (2 kg) fresh leave of *C. benghalensis L*. were collected in February 2024 from the inside of Adama Science and Technology University campus, which is found in Adama city of East Shewa zone of Oromia Regional State, Ethiopia. The plant material was identified in the National Herbarium, Addis Ababa University.

Figure 4: Morphological view of *C. benghalensis*

3.4 Preparation of *C. benghalensis* leaf extracts

After collection, the plant samples were washed repeatedly first with tap water and then with distilled water, and were allowed to air dry for over two weeks at room temperature without direct exposure to sunlight. Thereafter, the dried plant materials were grinded using an electric blender and the resulting powders were stored in a polyethylene bag to avoid certain environmental conditions (moisture, air, and other surrounding dust) until required for further use. The leaf powder was weighted to 300 g using an electronic balance, and it was soaked (macerated) in 1500 mL of methanol, petroleum ether, and chloroform separately for 72 hours with an electrical shaker at 120rpm. The plant extracts were filtered separately using Whatman No.1 filter paper. Then the filtrates were evaporated using a rotary evaporator, and the dried extracts (crudes) were stored in a refrigerator until used (Alade & Irobi, 1993).

Extraction yield (%) = Weight of the crude extract/Weight of the powdered sample ∗100.

Figure 5: Preparation of plant extracts (Crudes) A. Fresh leave B. collected and washed leave C. Dried at room temperature, D. Grinded plant sample E. Electrical shaker F. preparation of filtrates, G. Rota evaporator, H. extracted crudes.

3.5 Phytochemical analysis of *C. benghalensis L*. leave extracts

Petroleum ether, methanol and chloroform leaves crude extracts of *C. benghalensis L* was used to detect for the presence of secondary plant metabolites including, steroids, tannins, alkaloids saponins, phenols, flavonoids and terpenoids using standard methods with some modification (Samuel & Kumar, 2016b). The color intensity or the precipitate formation was used as analytical responses to these tests (Cross & Science, 2019).

Test for flavonoids: 3mg of extracts was treated with conc. H_2SO_4 solution. The formation of a yellowish orange color indicates the presence of flavonoids.

Test for saponins (Foam test): 3mg of extracts was diluted with 5mL of distilled water, shake vigorously and was observed for a stable persistent froth.

Test for tannins: To a 3mg of extracts, a few drops of 10% ferric chloride solution were added. The appearance of a green or blue color indicates the presence of tannins.

Test for steroids: 3mg of extract was mixed with 1mL of chloroform and 2-3 drops of conc. H_2SO_4 was added to it. The appearance of a pink or red color indicates the presence of steroids.

Test for Terpenoids (Salkowski test*)*: 3mg extracts were mixed with 2mL of chloroform and 3mL of conc. H_2SO_4 solution. A reddish-brown color at the interphase indicates the presence of terpenoids.

Test for alkaloids (Wagner' test): 3mg of the extract was treated with diluted HCl, filtered followed by addition of Wagner's reagent (1.27 g of iodine and 2g of KI along with 100mL of distilled water) and observed for the formation of reddish-brown colored precipitate.

Test for coumarins: 1.5ml of the extract was mixed with few drops of alcoholic sodium hydroxide in the watch glass; the appearance of yellow colour indicated the presence of coumarin.

3.6 Extraction of essential oil from fresh leaf of *C. benghalensis*

The fresh leaf of *c. bengalensis L.* (100 g) was hydro-distilled with 300 mL deionized water for about 2 hr using a modified Clevenger-type apparatus, following the methodology described by Esmaeili *et al* (2018). The condensate (mixture of essential oil and water) was collected in a 1000 mL separatory funnel. The essential oil was consecutively separated from the aqueous layer, and the separated EOs were then dried and kept in a sealed glass vial in the refrigerator for further experiment and analysis (Esmaeili *et a*l., 2018).

Figure 6: The essential oil extraction process from fresh leaves of C. benghalensis L. a) fresh leaf, b. indicated hydrodistlation, C. Filtration of oil extract and d. indicated funnel separation process of oil and water part.

3.6 Gas chromatography/mass spectrometry (GC-MS) analysis of the essential oils

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extracts of C. *benghalensis* were performed using a GC-MS (Model; Thermo GC –Trace Ultra) equipped with a VF 5ms fused silica capillary column of 30m length, diameter 0.25µm film thickness. The column over temperature was programmed from 70^{o}C to 260^{o}C for 6^{o}C/min. Ionization of the sample components was performed in electron impact mode (EI, 70 Ev). Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.0ml min-1. The mass range from 40-1000 m/z was scanned at a rate of 3.0 scans/s. 1.0µL of the methanol extract of *C. benghalensis* was injected to the GC-MS manually for analysis. Total running time of GC-MS is 40min. The relative percentage of the each extract constituents was expressed as percentage with pack area normalization (Malarvizhi *et al*., 2019).

3.7 Isolation of Bioactive Compound

3.7.1 Thin Layer Chromatography (TLC) analysis of extracts

The TLC plates supplied by Merck, Germany (TLC Silica gel 60 F254) was used to observe the separation of individual compound as a single spot will trimmed and the position of the origin marked by a straight line (Chioma & Omoregie, 2010).

Rf (Retention factor) = Distance travelled by substance / Distance travelled by solvent front (Chioma & Omoregie, 2010).

3.7.2 Column Chromatography

Methanol and chloroform crude extracts (25 g) of *C. benghalensis* leaf were adsorbed on 25 g of silica gel (mesh size 60-120) and subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity were used as the mobile phase (Arowora *et al*., 2019). The 150 g of Silica gel was mixed with 200 mL of n-hexane to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column, and the column was packed. Elution of the extract was done with increasing gradient of solvent systems (increasing gradient of ethyl acetate in n-hexane). The column was first eluted with n-hexane as the mobile phase, with the polarity increasing by 10 % increments of ethyl acetate followed by dichloromethane in methanol.

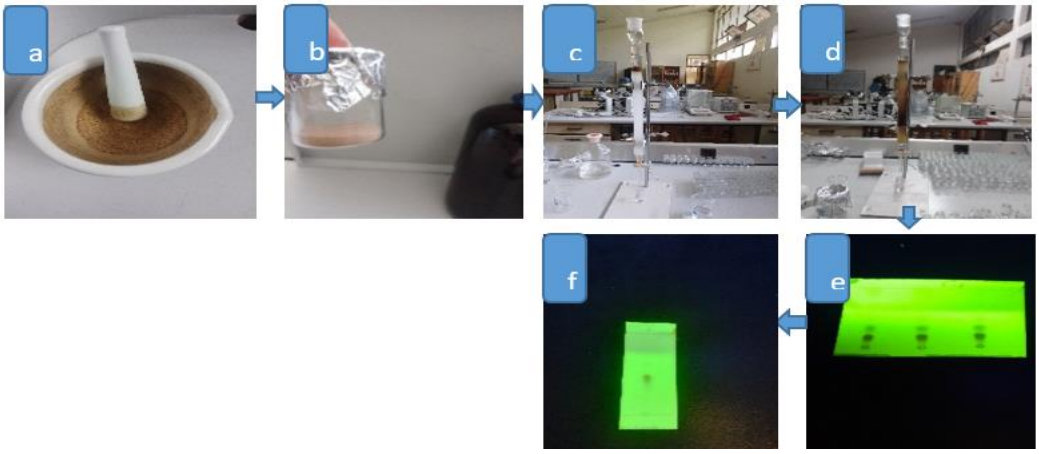


Figure 7: Overview of Column Chromatography and TLC tests. A. Mixing methanol and chloroform extracts B. mixed extracts C. Column packing D. Collection of fractions E, F and G are indicated the spots that appeared when TLC test under UV.

One hundred tweenty (120) fractions were collected and labeled, and the purity of all fractions was analyzed with TLC plates. Fractions that showed similar Retention fraction values and the same characteristic color on Thin-Layer Chromatography were combined. Fractions 25, 34,92 and 112 were mixed.

Table 1: Summary of fractionation of mixed extracts *C. benghalensis* leaf

Solveny sytstem	Ratio	Fraction	Solveny sytstem	Ratio	Fraction
n-hexane	10	F1-F3	Dichloromethane	10	F57-F59
n-hexane: EtOAc	9.5:0.5	F4-F5	Dichloro:methane	9.5:0.5	F60-F62
"	9:1	F6-F8	"	9:1	F63- F65
"	8.5:1.5	F9- F10	"	8.5:1.5	F66- F68
"	8:2	F11- F14	"	8:2	F69-F70
"	7.5:2.5	F15- F16	"	7.5:2.5	F71-F73
"	7:3	F17- F20	"	7:3	F74- F76
"	6.5:3.5	F21- F23	"	6.5:3.5	F77- F80
"	6:4	F24- 25	"	6:4	F81- F84
"	5.5:4.5	F26- F28	"	5.5:4.5	F85- 87
"	5:5	F29- F31	"	5:5	F88- F92
"	4.5:5.5	F32- F33	"	4.5:5.5	F91- F93
"	4:6	F34	"	4:6	F94- F96
"	3.5:6.5	F35- F37	"	3.5:6.5	F97- F99
"	3:7	F38- F39	"	3:7	F100- F102
"	2.5:7.5	F40- F42	"	2.5:7.5	F103- F107

“	2:8	F43- F44	“	2:8	F108- F111
“	1.5:8.5	F45- F47	“	1.5:8.5	F112- F114
“	1:9	F48- F51	“	1:9	F115- 116
“	0.5:9.5	F52- 53	“	0.5:9.5	F117- F118
“	0:10	F54- F56	“	0:10	F119- F120

EtOAc= ethyl acetate F= Fraction

3.7.3 Characterization of compound

The Compound was isolated from the methanol soluble fractions of the mixed extracts of the leave part of *C. benghalensis* by chromatographic separation and purification over silica gel (Cross & Science, 2019). The NMR spectroscopic technique (1H NMR, 13C NMR, and DEPT-135) spectra were employed to elucidate the structure of a compound (Arowora *et al*., 2019).

3.8. Biological activity

3.8.1. Antibacterial and antifungal activity

In vitro antibacterial activities of *C. benghalensis* leaf crude extracts and oil extract were examined. The antibacterial activities were determined against four pathogenic bacterial strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Streptococcus pyogenes* (ATCC-19615) by the agar disk diffusion method. The four sets of dilutions (200, 100, 50, and 25mg/ml) of *C. benghalensis* leaf crude extract and oil extract (50, 25, 12.5 6.25 mg/ml) were prepared. The strains of bacteria cells were adjusted at 1.5×10^8 colony forming units (CFU/ml). To determine the amount of bacteria present, a 0.5 McFarland standard was prepared by combining 0.05ml of 1 percent BaCl2 and 9.95ml of 1 percent H2SO4 in distilled water. To contrast a bacterial solution, the preparation was stored in a flask. Mueller-Hinton sterile agar plates were inoculated with indicator bacterial strains.

Sterile filter paper disks (Whatman No. 1, diameter = 6 mm) were placed on the inoculated Mueller-Hinton agar plates. From each dilution 20µL were taken and dispensed on the filter papers and allowed to stay at 37°C for 24 hours. Control experiments were carried out under similar

condition by using Ciprofloxacin as positive control and DMSO as negative control. The zones of growth inhibition around the disks were measured after 18 to 24 hours. The sensitivities of the bacterial strains to the leaf extracts were determined by measuring the sizes of inhibitory zones starting from the edge of disk to the edge of the clear zone on the agar surface around the disks, and values <7 mm were considered as not active against microorganisms.

Similarly, the fungus (*C. albicans ATCC* 10231) was sub-cultured on SDA and incubated for 72 h at 27℃. The inoculum suspension was prepared by picking two up to three colonies of the fresh cultures of SDA with a sterile inoculating loop and transferred into a test tube containing 10 ml of SDB and incubated overnight. Then, new cultures were diluted with a saline solution and vortexed thoroughly. Finally, the fungal isolate was matched with 0.5 McFarland standards corresponding to 1×10^6 cells for fungal species.

3.8.2. Disk Diffusion Method

The *in vitro*, disc diffusion method was conducted to evaluate the antimicrobial activities of *C. benghalensis leaf* crude extracts and extracted essential oil against microbial strains following the methods of (Al-Shahrani & Belali, 2024). For this experiment, Muller-Hinton agar (MHA) (for bacteria) and Sabouraud dextrose agar (SDA) (for fungus) media were prepared and autoclaved at 121℃ for 15 psi. After autoclaving, media was poured into sterile petri plates up to a uniform thickness of approximately 4 mm and allowed to solidify. As mentioned above, the microbial inoculum was subculture into the MHB for bacteria and SDB for fungus and incubated overnight for fresh culture. Then, these inoculums were diluted to adjust with 0.5 McFarland standards. A sterile cotton swab was inserted into the culture suspension to test bacteria and fungi. The swab was then streaked on the surface of the Muller-Hinton agar and SDA plate for bacterial strains and *C. albicans* 10231^T (ATCC), respectively. The swab was streaked three times over the entire plate surface to ensure a uniform, confluent growth. A 200 mg/ml of stock solution of each crude extract was prepared by dissolving 0.4 g of methanol, chloroform and petroleum in 2 ml of 10% DMSO. Each extract was formed for disc diffusion assay by two-fold serial dilution (00, 250, 125, and 62.5 mg/ml). Two- fold serial dilution (200, 100, 50, and 25 mg/ml) were formed from this stock solution. The sterilized filter paper (Whatman No. 1, diameter = 6 mm) disc was dipped in the extracts. Then, the extracts and essential oil were left to diffuse in the disc for 30 min and placed on the inoculated agar (El-

bashiti *et al*., 2017). Sterilized paper discs soaked in 10 µg/ml ciprofloxacin and ketoconazole standardized antibiotic were used as a positive control. At the same time, a disc impregnated with DMSO was used as a negative control. The plates were incubated overnight at 37 ℃ for 24 h for bacterial and fungal at 25 ℃ for 48 h. Finally, the microbes' inhibition zone by microbial agents was measured using ruler in mm scale. The test was done in triplicate, and the results were presented as an arithmetic average mean (mean ±SD).

3.8.3. Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Broth dilution method was adopted to find minimum inhibitory concentrations (MICs) of active extracts. *C. benghalensis* crude extracts and oil that exhibited antibacterial activity in the preceding test were chosen. The initial concentration of the crude (5mg/ml) and essential oil (5 mg/ml) were diluted using two-fold serial dilution by transferring 1 ml of crude into 1 ml of sterile nutrient broth and mixed it into a vial, and then it was serially diluting it into 5 vials. Each concentration was inoculated with 0.02 ml of the standardized bacterial cell suspension and incubated for 24 hours at 37 ^{0}C. The turbidity or cloudiness of the broth was indicator of bacteria's growth in the broth (Rodr *et al.*, 2022).

3.8.4 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

From each of the test tubes in the MIC determination that did not show any visible growth, 50µl of the broth was aseptically inoculated onto a sterile Mueller Hinton agar (MHA) surface for bacteria and Sabouraud dextrose agar (SDA) surface for fungus and gently spread all over the surfaces with a sterile bent glass rod. The inoculated plates were incubated for 24 h at 37℃ for bacteria and at 25℃ for 48 h for fungus. After incubation, the MBC and MFC were regarded as the dilution at which the plate had no visible growth (Maharjan et al., 2011).

3.9 Antioxidant activity of crude extracts and Essential Oil

Antioxidant activity of the three crude extracts (methanol, chloroform and petroleum ether) and Essential oil were measured based on their free radical scavenging activity, which was determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. The extracts were dissolved in five vials containing methanol to give 1000, 500, 250, 125 and 62.5 µg/mL. Ascorbic acid was used as a

positive control. Sample free DPPH solution in methanol was used as negative control. The solution of DPPH (0.04 mg/mL) in methanol was prepared, and 4 mL of this solution was mixed with 1 mL of extract solution at various concentrations immediately and then incubated in dark for 30 min at room temperature to complete the reaction. The absorbance of the sample was measured at 517 nm (Kalia *et al*., 2021). Radical scavenging activity was expressed as the inhibition percentage (IP) of free radical and was calculated using the formula:

$$IP\ (\%) = ([Acontrol - Atest)/Acontrol]) \times 100\%$$

Where a control is the absorbance of the control reaction (containing all reagents except the tested extracts), and A test is the absorbance of the test extract (Suprava *et al*., 2012). The percentage DPPH inhibition was calculated according to the following formula,

% Radical scavenging activity= [(absorbance of control-absorbance of sample)/control]x100

The IC50 values were calculated by plotting DPPH percentage inhibition versus log transformed concentration of sample extract.

3.10. Data analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data was subjected statistical Package of Social Science (SPSS 26.0) version was used to analyze the experiment results and the results were presented using tables and graphs. The difference between means were determined by using one-way ANOVA and least significant difference test. The difference between means were determined by using one-way ANOVA and least significant difference test. The level of statistical significance was set at $p \leq 0.05$. Microsoft Excel 2016 statistical package were used for all analyses.

CHAPTER-4

RESULTS AND DISCUSSIONS

4.1 The yield of the plant extract

The extraction yield measures the solvent efficiency to extract specific components from the original material. In this study the *C. benghalenses L*. leaf crude extraction was done with three different organic solvents, such as methanol, chloroform and petroleum ether (Table 2). 300 g of the plant powder was used in all solvents respectively. The mass of extract that was obtained was different in all of three solvents. The methanol yielded 23.54 g in highest amount regarding to left solvents, chloroform is yielded with the amount of 5.11g and petroleum 2.62g which is the minimum amount in the present experiment.

Table 2: Yield of Different Extracts of *Commelina benghalensis* L. leaf

Solvents	Initial mass of the plant(g)	Yield of extract (g)	Percentage (%) yield	Color of the crude
Methanol	300	23.54	75.27	Greenish yellow
Chloroform	300	5.11	16.34	Greenish yellow
Petroleum	300	2.62	8.37	Greenish yellow

Successful determination of biologically active compounds from plant material was largely depended on the type of solvent used in the extraction procedure (Pandey & Tripathi, 2014). Methanol is often used due to its ability to extract a wide range of polar and non-polar compounds. Studies indicated that methanolic extracts yielded significant amounts of bioactive compounds, making them highly effective for pharmacological applications (Cuéllar Cuéllar & Okori, 2010). According to Cuéllar & Okori (2010), chloroform was a moderately polar solvent that can extract specific phytochemicals that are less soluble in water but more soluble than in non-polar solvents. Petroleum ether is primarily used for extracting non-polar compounds. Understanding the

solvents' characteristics helps in optimizing extraction methods for desired phytochemicals from plant parts.

According to Liu *et al*. (2022), a multi-step solvent partitioning method was used to isolate specific compounds from *Commelina* species. The process began with the extraction of 400g of the aerial parts of *Commelina* sp. using 1100 mL of methanol at room temperature, which was then concentrated to yield 2.6g of crude extract (Liu *et al.*, 2022). Samuel *et al*. (2016) conducted plant extraction different solvent extracts from the leaves and stems of *C. benghalensis L*. The plant material was extracted using methanol, aqueous solution, carbon tetrachloride (CCl_4), and hexane (Samuel *et al*., 2016b).

4.2 Phytochemical screening

Phytochemicals are essential for the defense mechanisms of plants. They help protect against stress from interactions between different species. Additionally, phytochemicals serve as important reducing agents in the synthesis of antimicrobial compounds. These substances have been utilized in traditional medicine for their beneficial properties (Malarvizhi *et al.*, 2019). To reveal this, in current study the extract of *C. benghalensis* leave was subjected to qualitative screening of phytochemicals. The results of phytochemicals screening of the methanol extract was positive for almost about all phytochemical tests and revealed the presence of secondary metabolites. The terpenoids and saponins constituents and courmarins present in chloroform extract while the others absent. In the petroleum extract, tannin, steroids and phenols are appeared. The phytochemical analysis of methanol, chloroform and petroleum ether extracts of *C. benghalensis* are shown in Table 3 which indicates the presence of medicinally active compounds such as namely alkaloids, tannin and coumarins, steroids, terpenoids, phenols, flavonoids and saponins. Studies have shown that these compounds exhibit several pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Muniyandi *et al.*, 2018).

In the current study, qualitative analysis of phytochemicals in *C. benghalensis L*. revealed that alkaloids were detected only in the chloroform extract, indicating a potential for specific bioactivity associated with this group. Tannins were present in the methanol extract but absent in chloroform, suggesting that methanol is more effective for extracting tannins from this plant. Flavonoids were found in both methanol and chloroform extracts, highlighting their potential health benefits and antioxidant properties. Steroids were exclusively present in the petroleum

extract, which may indicate a unique profile of steroid compounds that could be explored for medicinal applications. Terpenoids were detected in both methanol and chloroform extracts, suggesting their significant role in the plant's chemical composition. Saponins were present in both methanol and chloroform extracts, indicating their potential use in traditional medicine due to their known health benefits. Phenols were found in the methanol extract but absent in chloroform, emphasizing methanol's efficacy in extracting phenolic compounds. Lastly, coumarins were present in both methanol and chloroform extracts, which contributes to the plant's therapeutic properties.

Table 3: Preliminary phytochemical screening of *C. benghalensis L*. (Leaf) extracts

	Extracts		
Phytochemicals	Methanol	Chloroform	Petroleum ether
Alkaloids	-	+	-
Tannin	+	-	+
Flavonoids	+	+	-
Steroids	-	-	+
Terpenoids	+	+	-
Saponins	+	+	-
Phenols	+	-	+
courmarins	+	+	-

+= present -=absence

The other study reported by Tadesse & Ganesan, 2016b revealed that the methanol extracts *C. b* have been shown to contain a wide range of phytochemicals including alkaloids, flavonoids, saponins, tannins, phenols, and glycosides. These compounds are known for their antioxidant and antimicrobial properties (Tadesse & Ganesan, 2016b). On the other hand, the methanolic extract

demonstrated significant antioxidant activity, making it useful in combating oxidative stress-related diseases (Harini & Jayanthi, 2022). The authors indicated that the petroleum ether extract of *C. benghalensis* contained phytochemicals, including phenols and flavonoids, which are recognized for their insecticidal effects (Tâm *et al*., 2016). Previously some study reveals that chloroform extracts of this plant primarily contain alkaloids, flavonoids, and saponins, though the overall yield of bioactive compounds was generally lower compared to methanol extract. The presence of these compounds suggests potential therapeutic benefits. chloroform extract has been noted for its effectiveness in isolating specific non-polar compounds that may have medicinal properties (Purushothaman & Sumithra, 2017).

The presence of alkaloids is consistently reported across multiple studies. For instance, Krishna Satya *et al.* (2016) noted alkaloids in both chloroform and aqueous extracts but found a lack of phenols and terpenoids in the chloroform extract. Flavonoids were also identified in various studies, including one that utilized gas chromatography-mass spectrometry (GC-MS), revealed bioactive compounds such as phenolic acids and flavonoids (Adolph, 2016; Harini & Jayanthi, 2022). Saponins were found in the methanol extract in the current study, corroborating findings by Balakrishnan *et al*. (2015), who reported saponins alongside other compounds like catechins and glycosides in their analyses. The presence of tannins was noted as well, which were recognized for their antioxidant properties. The diverse range of phytochemicals identified *in C. benghalensis* suggests its potential as a source for developing new therapeutic agents. The documented antioxidant properties linked to flavonoids and tannins could be harnessed for treating oxidative stress-related conditions (Tadesse & Ganesan, 2016a). Furthermore, the presence of saponins indicates possible cardiovascular benefits, aligning with traditional uses in herbal medicine for managing heart-related ailments (Adolph, 2016). The qualitative phytochemical analysis of *C. benghalensis* revealed a complex array of bioactive compounds that can contribute to its medicinal value. While there are consistent findings regarding key constituents like alkaloids and flavonoids across various studies, differences in compound presence based on extraction methods underscore the importance of methodological choices in phytochemical research.

4.3 Essential oil of *Commelina benghalensis L.*

In this study, the methanol extract and hydro-distillated essential oil of the fresh leave of the plant were analyzed. During the GC-MS analyses, the excellent separations achieved by the GC permitted the inspection of each of the peaks. The chemical composition of the Essential Oils (Eos) from *C. benghalensis L*. leaves were listed in **Table 4**. The extract of Essential Oils of the *C. benghalensis L.* was light green and had a specific odor. GC-MS analysis of essential oils from leaves of *C. benghalensis L*. revealed a total of 25 chemical components, accounting for 76.05 % of the total compositions, the major constitutes were Di(2-ethylhexyl) phthalate (28.74 %), Dimethyl Sulfoxide (22.66%), Davanone (13.11%), 4-Cyclohexylphenol (6.13%) and 2,4-Di-tert-butylphenol (5.41%). The remaining constituents ranged from 0.67 % to 3.34 %.

Table 4: GC-MS analysis of essential oil of C. *benghalensis L.* (fresh leaf)

Compound Name	**RT**	**Formula**	**Composition %**
Dimethyl Sulfoxide	3.175	C_2H_6OS	22.66
Trans-3-hexen-1-ol	3.264	$C_6H_{12}O$	1.58
Styrene	3.691	C_8H_8	0.67
1,1,2,2-tetrachloroethane	3.97	$C_2H_2Cl_4$	3.34
Carbolic Acid	5.029	C_6H_6O	0.75
1,1,2-trimethyl-3-(2-methyl-1-propenyl)cyclopropane	5.728	$C_{10}H_{18}$	0.81
Dodecane	6.434	$C_{12}H_{26}$	0.64
Dodec-1-ene	9.292	$C_{12}H_{24}$	1.09
2-Methyldecane	11.24	$C_{11}H_{24}$	0.73
4-Cyclohexylphenol	11.729	$C_{12}H_{16}O$	1.98
4-Cyclohexylphenol	12.279	$C_{12}H_{16}O$	6.13
Dodecyl Alcohol	13.657	$C_{12}H_{26}O$	2.15
Ethy Cinnamate	15.28	$C_{11}H_{12}O_2$	0.67
Hydroxyphenylmethyl ketone	15.809	$C_{19}H_{22}O_2$	0.73
2,4-Di-tert-butylphenol	16.196	$C_{14}H_{22}O$	5.41
5-Isopropyl-1,2,3,4-tetramethylbenzene	16.99	$C_{13}H_{20}$	0.67
Viridiflorol	17.221	$C_{15}H_{26}O$	0.6

Davanone	17.729	$C_{15}H_{24}O_2$	13.11
Ferruginol	21.315	$C_{18}H_{26}O$	0.72
Cyclohexadecane	21.39	$C_{16}H_{32}$	3.25
Germacrene D	21.967	$C_{14}H_{20}$	0.75
DBP	24.309	$C_{16}H_{22}O_4$	1.12
3-Bromobenzoic acid methyl ester	26.114	$C_{14}H_{11}BrO_2$	0.9
Di(2-ethylhexyl) phthalate	32.734	$C_{24}H_{38}O_4$	28.74
1-Octadecanol	33.216	$C_{18}H_{38}O$	0.81
			100.01

RT=Retention time

The major constituents mentioned—Di(2-ethylhexyl) phthalate, Dimethyl Sulfoxide, Davanone, 4-Cyclohexylphenol, and 2,4-Di-tert-butylphenol—were main diverse organic compounds detected from *C. benghalensis* in current study. Di(2-ethylhexyl) phthalate (DEHP) is a colorless, viscous liquid that is slightly soluble in water but highly soluble in organic solvents. It has a high boiling point and a low vapor pressure, making it stable under various conditions. This compound is used as a plasticizer in PVC products (e.g., toys, medical devices, flooring) and other polymers. Also utilized in hydraulic fluids, dielectric fluids in capacitors, and as a solvent in various formulations (Identity, 2013)

Dimethyl Sulfoxide (DMSO) is a polar aprotic solvent with the formula $CH_3)_2SO$ $CH_3)_2SO$. It is known for its ability to penetrate biological membranes and has a high boiling point. Used widely as a solvent in chemical reactions, pharmaceuticals, and as a cryoprotectant in biological samples. It also acts as a radical scavenger and has anti-inflammatory properties. Scientific papers showed that, Davanone is an organic compound derived from the essential oil of certain plants (Coleman *et al.*, 2007). Known for its use in perfumery due to its pleasant fragrance and potential therapeutic properties. In industrial contexts, davanone serves as a surfactant and emulsifier, making it

valuable in food processing and cosmetic formulations. It`s properties allow it to enhance product stability and improve texture (Coleman *et al*., 2007; Tesfaye et a*l*., 2020).

Cyclohexylphenol (CHP) is an organic compound with notable applications and characteristics. CHP is widely used in the preparation of dyes and resins, making it valuable in the chemical manufacturing industry. It is also utilized in the formulation of biocides, which are essential for pest control and preservation. Cyclohexylphenol serves as a biochemical tool in proteomics for studying protein interactions and functions due to its ability to act as a substrate for various enzymes. Researches indicated that CHP exhibits estrogenic activity, activating olfactory receptors involved in hormone signaling pathways, suggesting potential implications in endocrine studies. Cyclohexylphenol is a versatile compound with significant industrial and biochemical applications (Gutierrez-Rubio *et al*., 2021). The other from main compounds of extracted from *C. benghalensis* is 2,4-Di-tert-butylphenol, which is a phenolic compound characterized by two tert-butyl groups attached to the benzene ring at the 2 and 4 positions. It is known for its antioxidant properties and is used in various industrial applications, including as a stabilizer in plastics and as an ingredient in personal care products (Choi *et al*., 2013).

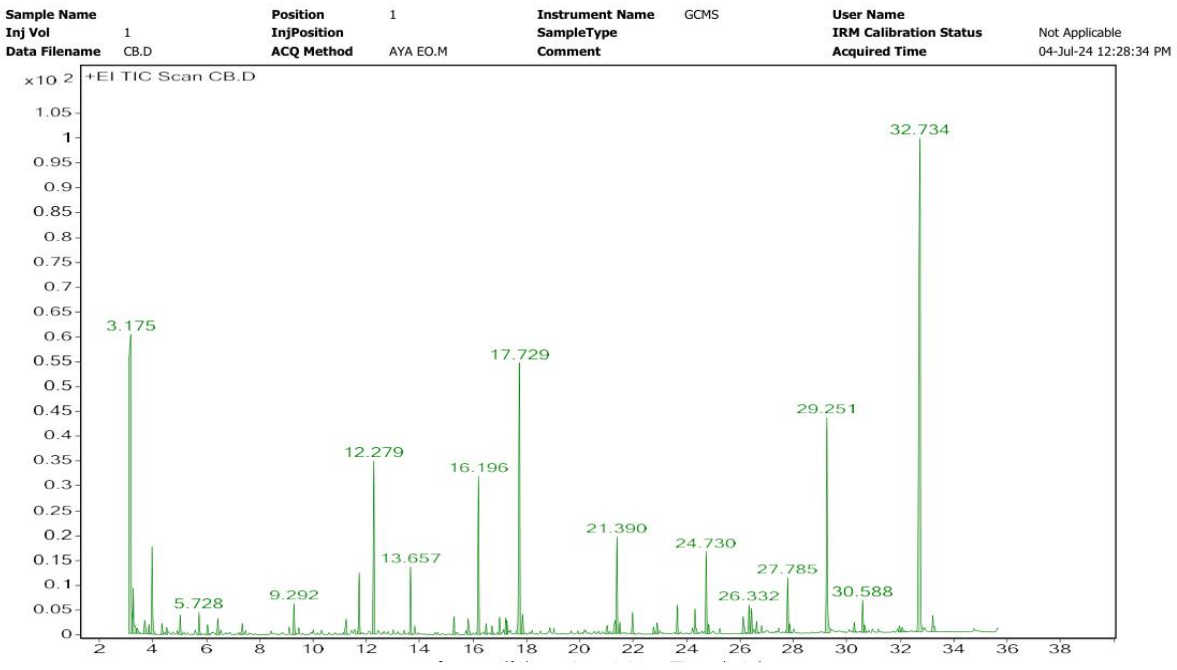


Figure 8: Chromatogram showing peaks for bioactive compounds in the leaf extract of C. benghalensis L.

These compounds from *C. benghalensis* illustrate the plant's chemical diversity and its potential applications across various fields, including pharmaceuticals, materials science, and environmental health. Their unique properties contribute to ongoing research into their benefits and risks, particularly concerning human health and ecological impacts. From another study, methanolic extract *C. benghalensis* 19 bioactive compounds reported according to GC-MS analyses (Kansagara & Pandya, 2023).

4.4 Isolation of Bioactive Compound

4.4.1 Thin Layer Chromatography (TLC) analysis of extracts

The column chromatographic fractions obtained from mixed methanol and chloroform extract was analyzed using TLC with the results depicted in Appendix 11. The results of the TLC profile demonstrated the presence of the same spots which confirmed the existence of the single compound in the extract. Therefore, the dichloromethane extract was subjected to silica gel column chromatography.

4.4.2 Characterization of compound

During compound isolation, most collected fractions were colorless, indicating a potentially low concentration of the target bioactive compound. To address these, fractions were analyzed by UV visualization to identify the containing single compound, leads to the combination of fractions 24, 25, 38, 82, and 92 (n-hexane: EtOAc 6:4 and 3:7, Dichloro:methane 6:4 and 5:5 respectively) based on matching UV spot characteristics. Subsequent bioactive characterization of this combined fraction using NMR and GC-MS revealed the presence of 1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester (Figure 9). 1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester, more commonly known as Bis (2-ethylhexyl) phthalate (DEHP), is a powerful plasticizer. This phthalate ester presents as a colorless, nearly odorless, oily liquid, chemical formula $C_{24}H_{38}O_4$ and has molecular weight of 390.56 g/mol (Den Braver-Sewradj *et al.*, 2020). DEHP is primarily used to impart flexibility and pliability to plastics, especially polyvinyl chloride, also it has been used in medical equipment and food packaging (Rowdhwal & Chen, 2018). DEHP can be found in various products, including wall coverings, tablecloths, floor tiles, and furniture upholstery (Agency, 2002).

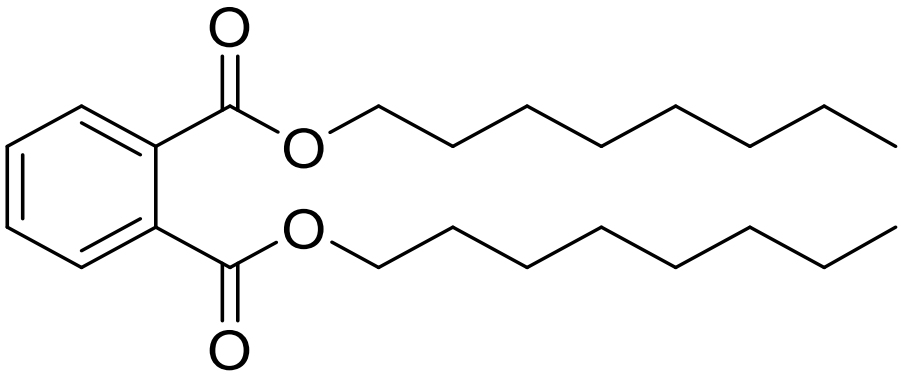


Figure 9: 1,2-benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester.

A study by Khatun *et al*. (2019) highlighted that *C. benghalensis* had been extensively investigated for its phytochemical properties, resulting in the isolation of several bioactive compounds, including Dammara-12-en-3-one (CB-1), stigmasterol (CB-2), and 3-(2,3,4,5,6-pentahydroxy)-cinnamoyl dammara-12-ene (CB-3), from the n-hexane fraction of its methanol extract (Khatun *et al.*, 2019). Research by Kumar et al. (2023) focused on developing formulations from *C. benghalensis* extracts, identifying various bioactive compounds with antioxidant, antibacterial, and analgesic properties. The study emphasized the plant's potential for pharmaceutical applications based on its rich phytochemical profile (Khanpara & Pandya, 2023).

4.5 Biological activity of crude extracts and essential oil *of C. benghalensis L.*

4.5.1 Antimicrobial activity of C. *benghalensis* extracts (crudes and essential oil)

The extracts (methanol, chloroform and petroleun ether) and essential oil of the C. *benghalensis* were tested for their antimicrobial activity in different concentrations against two Gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains and fungal strain (*C. albicans ATCC* 10231) compared to as the positive control ciprofloxacin and Ketoconazole and DMSO was used as a negative control respectively for bacteria and fungi. Overall antibacterial activities of *C. benghalensis* extracts based on the zone of inhibition ranged between 6±0.6mm and 15.7±1.5mm and antifungal activity of C. *benghalensis* extracts ranged 8.7±1.2mm and 16.7±3.1mm (Table 5).

The effectiveness of plant extracts against *E. coli* (Appendix 1) is evaluated through p-values, with values below 0.05 indicating significance. Ciprooxacin significantly outperforms Petroleum and methanol with p-values of 0.000, while EO also shows significant superiority over Petroleum. In

contrast, comparisons like methanol vs chloroform and EO vs methanol yield p-values above 0.05, indicating no significant differences. Overall, Ciprooxacin and EO exhibit higher antibacterial activity compared to Petroleum and methanol (Valgas *et al.*, 2007). The p-values from the Tukey HSD test (Appendix 2) revealed significant differences in inhibition zones among plant extracts against *S. aureus*. Significant comparisons, marked by p-values of 0.000, include PC (ciprooxacin) vs methanol, chloroform, Petroleum and EO, indicated that ciprooxacin significantly outperforms these extracts. The comparison like methanol vs chloroform, Petroleum vs methanol and chloroform and EO vs methanol and chloroform show p-values greater than 0.05, suggesting no significant differences in inhibition between these extracts. Overall, ciprooxacin demonstrates superior antibacterial activity compared to the other extracts tested (Silva *et al*., 2011). The p-values from the Tukey HSD test reveal significant differences in inhibition zones among plant extracts against *P. aeruginosa* (Appendix 3). Ciprooxacin shows significant superiority over all other extracts with p-values of 0.000, indicating a substantial difference in inhibition efficacy. EO also exhibits significant differences against Methanol and chloroform with p-values of 0.022 and 0.007, respectively. In contrast, comparisons like Methanol Vs Chloroform and Petroleum vs chloroform yield p-values above 0.05, indicating no significant differences. Overall, ciprooxacin demonstrates the highest antibacterial activity, followed by EO, which also shows significant effects against some extracts.

The p-values from the Tukey HSD test revealed significant differences in inhibition zones among plant extracts against *S. pyogenes* (Appendix 4). Ciprooxacin shows significant superiority over all other extracts with p-values of 0.000, indicating a substantial difference in inhibition efficacy. Petroleum and EO also exhibit significant differences against certain extracts, with p-values of 0.004 and 0.000, respectively. In contrast, comparisons like methanol vs chloroform and EO vs Methanol yield p-values above 0.05, indicating no significant differences. Overall, ciprooxacin demonstrates the highest antibacterial activity.

Table 5: Antibacterial activity (zone of inhibition) of methanol, chloroform, petroleum and essential oil of *C. benghalensis L.*

S.N	Measurable zone of inhibition of Methanol extract (mm)								
	Concentration (mg/mL)	*E. coli* ATTC 25922^{T}	%RI	*S.aureus* ATCC 25923^{T}	%RI	*P.aeruginosa* ATCC 27853^{T}	%RI	*S.pyogene* ATCC 12204^{T}	%RI
1.	200	12±0.6^{b}	49.4	14±1.7^{c}	60.1	12.3±1.5^{b}	44.4	14.3±0.6^{b}	53.6
2.	100	11±0^{ab}	45.3	10.6±0.6^{b}	45.5	10±1^{ab}	36.1	11±1.7^{ab}	41.2
3.	50	9.3±0.6^{a}	38.3	10±1^{b}	42.9	9±0^{a}	32.5	9.7±0.6^{a}	36.3
4.	25	8.3±0.6^{a}	34.2	7.7±0.6^{a}	33	8±0^{a}	28.9	9±1^{a}	33.7
5.	PC	24.3±2^{c}	100	23.3±0.6^{d}	100	27.7±2.1^{c}	100	26.7±0.6^{c}	100

PC= positive control, %IR= Resistance Index

S.N.	Measurable zone of inhibition of Chloroform extract (mm)								
	Concentration (mg/mL)	*E. coli* ATTC 25922^{T}	%RI	*S. aureus* ATCC 25923^{T}	%RI	*P.aeruginosa* ATCC 27853^{T}	%RI	*S.pyogene* ATCC 12204^{T}	%RI
6.	200	10±1^{b}	38.9	14±1^{c}	58.3	11.3±1.5^{a}	43.5	12±1^{c}	46.2
7.	100	9±1^{ab}	35.0	11.7±0.6^{b}	48.8	9.7±0.6^{a}	37.3	9.3±0.6^{b}	35.8
8.	50	8.3±0.6^{ab}	32.3	9.7±0.6^{ab}	40.4	9.3±0.6^{a}	35.8	8±0^{ab}	30.8
9.	25	7±0^{a}	27.2	8.3±0.6^{a}	34.6	7.7±0.6^{a}	29.6	7.3±0.6^{a}	28.1
10.	PC	25.7±1.2^{c}	100	24±1^{d}	100	26±2.6^{b}	100	26±1^{d}	100

PC=positive control, %IR= Resistance Index

S. N	Measurable zone of inhibition of Petroleum extract (mm)								
	Concentration(mg/mL)	*E. coli* ATTC 25922^{T}	%RI	*S. aureus* ATCC 25923^{T}	%RI	*P.aeruginosa* ATCC 27853^{T}	%RI	*S.pyogene* ATCC 12204^{T}	%RI
11.	200	9.3±0.6^{c}	39.9	12.7±1.16^{c}	57	9.3±1.23^{c}	34.4	9.7±0.6^{c}	38.8
12.	100	8.3±0.6^{bc}	35.6	10±0^{b}	44.8	8.7±0.6^{bc}	32.2	8.3±0.6^{bc}	33.2
13.	50	7.3±0.6^{ab}	31.3	8.3±0.6^{b}	37.2	7±0.6^{ab}	25.9	8±0^{b}	32
14.	25	NA^{a}		NA^{a}		NA^{a}		NA^{a}	
15.	PC	23.3±0.6^{d}	100	22.3±0.6^{d}	100	27±1^{d}	100	25±1^{d}	100

NA=no activity against test organism, PC= positive control, %IR= Resistance Index

S.N.	Measurable zone of inhibition of Essential Oil extract (mm)								
	Conc. (mg/mL)	*E. coli* ATTC 25922^{T}	%RI	*S. aureus* ATCC 25923^{T}	%RI	*P.aeruginosa* ATCC 27853^{T}	%RI	*S. pyogene* ATCC 12204^{T}	%RI
16.	50	14.7±1.5^{c}	63.9	15.7±1.5^{c}	65.4	15.7±1.5^{b}	61.1	15.3±1.5^{c}	65.7
17.	25	11.7±0.6^{b}	50.9	12.7±0.6^{b}	52.9	12.7±0.6^{ab}	49.4	12.7±1.5^{bc}	54.5
18.	12.5	10.3±0.6^{ab}	44.8	11.3±0.6^{b}	47.1	11. ±1^{a}	42.8	12±1^{ab}	51.5
19	6.25	8±0.6^{a}	34.8	8.7±0.6^{a}	36.3	9.7±0.6^{a}	37.7	9±1^{a}	38.6

20	PC	23±1[d]	100	24±1[d]	100	25.7±1.5[c]	100	23.3±0.6[d]	100

%IR= Resistance Index PC=positive control

The methanolic extract exhibited varying degrees of antibacterial activity against both Gram-negative (*E. coli, P. aeruginosa*) and Gram-positive *(S. aureus, S. pyogenes)* bacteria, with inhibition zones generally decreasing as the extract concentration decreases. At 200 mg/mL, the extract shows the highest inhibition zones, with *S. aureus* displaying the greatest susceptibility at 14±1.7 mm and *P. aeruginosa* showing the lowest at 12.3±1.5 mm, indicating a relatively stronger efficacy against Gram-positive pathogens compared to Gram-negative ones. Comparatively, the positive control (PC) demonstrates significantly higher antibacterial activity across all tested pathogens, underscoring its effectiveness as an antimicrobial agent.

The chloroform extract demonstrated varying antibacterial effectiveness against Gram-positive and Gram-negative pathogens, with the highest inhibition zone recorded for *Staphylococcus aureus* at 14±1 mm at a concentration of 200 mg/mL, indicating significant susceptibility among Gram-positive bacteria. Conversely, the lowest inhibition zone is observed for Escherichia coli at 10±1 mm, reflecting a relatively lower efficacy against this Gram-negative bacterium. In comparison, the positive control (PC) shows markedly higher inhibition zones across all tested pathogens, with the highest at 25.7±1.2 mm, underscored its superior antibacterial activity compared to the chloroform extract.

The methanol extract exhibited significant antibacterial activity, particularly against *S. aureus* and *E. coli*. At a concentration of 200 mg/mL, the ZOI for *S. aureus* was recorded at 14 mm, suggesting strong inhibitory effects. The chloroform extract also demonstrated antibacterial properties, with the highest ZOI against *S. aureus* at 14 mm at 200 mg/mL. However, its efficacy was slightly lower compared to the methanol extract, indicating that while chloroform is effective, it may not extract as many active compounds as methanol. The petroleum extract showed comparatively lower activity across all tested strains. The maximum ZOI recorded for S. aureus was 12.7 mm at 200 mg/mL, which is significantly less than that observed in both the methanol and chloroform extracts. This suggests that petroleum extracts may contain fewer bioactive compounds or that their solubility in petroleum is less effective for antibacterial action. The essential oil extract exhibited the highest antibacterial activity among all tested extracts. At a

concentration of 50 g/mL, it achieved a ZOI of 15.7 mm against *P. aeruginosa*, showcasing its potential as a potent antimicrobial agent. This indicates that essential oils may have unique compounds that are highly effective against bacterial pathogens.

A study conducted by Ahmed *et al*. (2002) assessed the antibacterial activity of a 95% ethanolic extract of *C. benghalensis*, along with its fractions, which included carbon tetrachloride, n-hexane, and chloroform (Ahmed *et al*., 2016). The extracts were tested against several gram-positive and gram-negative bacteria, showing significant inhibitory effects, particularly against *S. aureus* and *S. typhi*. The diameter of inhibition zones ranged from 6 mm to 28 mm, depending on the extract and bacterial strain used. The other study that focused on the antimicrobial properties of the leaf extract of *C. benghalensis* reported that the ethanol extract exhibited considerable activity against *C. albicans, E. coli*, and *S. aureus*, with varying degrees of effectiveness The results indicated that ethanol extracts were more potent compared to aqueous extracts, which lost their activity upon dilution (Cuéllar Cuéllar & Okori, 2010). According to a report by Adolph et al., (2016) the chloroform extract *of C. benghalensis* demonstrated notable antibacterial properties . It showed significant inhibition against various bacterial strains, with specific zones of inhibition reported for Gram-positive and Gram-negative bacteria. For instance, it exhibited a zone of inhibition of 11 mm against *S. aureus* (Adolph, 2016). The petroleum ether extract was generally showed less effectiveness when compared to chloroform and methanol extracts. While some studies indicated mild antibacterial activity, it did not consistently show significant inhibition against the tested bacterial strains (Adolph, 2016).

The results from this study underscore the potential of *C. benghalensis L*. as a source of natural antimicrobial agents, with variations in efficacy based on the extraction method used. The methanol and essential oil extracts showed the most promise against pathogenic bacteria, particularly S. aureus and E. coli. These findings align with previous literature highlighting the importance of solvent choice in extracting bioactive compounds from medicinal plants. The positive control demonstrates significantly higher zones of inhibition compared to the methanol, Chloroform, Petroleum and Essential Oil extracts, indicating that while the extracts have moderate antibacterial properties, it is less effective than standard antibiotics.

Table 5, presents the antifungal activity of *C. benghalensis* extracts using methanol, chloroform, and petroleum as solvents against *C. albicans*. The measurable zone of inhibition (in mm) indicates

the extent to which the extract inhibits the growth of *C. albicans.* A larger zone generally suggests higher antifungal activity. Different concentrations of the extracts (25, 50, 100, and 200 mg/mL) were tested to determine the dose-response relationship. This value, calculated as a percentage, likely represents the resistance index, possibly relative to the positive control. A lower % RI suggests a more effective inhibition of fungal growth. The positive control provides a baseline for comparison, representing 100% inhibition.

The p-values from the Tukey HSD test reveal significant differences in inhibition zones among plant extracts against *C. albicans* (**Appendix 5**). Ketoconazole shows significant superiority over all other extracts with p-values of 0.000, indicating a substantial difference in inhibition efficacy. In contrast, comparisons like methanol vs chloform, Petroleum vs methanol, and EO vs methanol yield p-values above 0.05, indicating no significant differences. Overall, ketoconazole demonstrated the highest antifungal activity.

Table 6: Antifungal activity (Zone of inhibition) of Methanol, Chloroform, Petroleum and Essential Oil of *C. benghalensis L.*

S.N.	Methanol extract (mm)		Chloroform extract (mm)		Petroleum extract (mm)	
	Conc. (mg/mL)	*C.albicans* ATCC 10231^T	%RI	*C.albicans* ATCC 10231^T	% RI	*C. albicans* ATCC 10231^T	%RI
1.	200	13.3 ± 1.5^b	62.4	14 ± 1^c	67.6	16.7 ± 3.1^{ab}	71.7
2.	100	13 ± 1^b	61	12 ± 1^{bc}	58	14 ± 3.5^{ab}	60.1
3.	50	11 ± 0.6^{ab}	51.6	10.7 ± 1.2^{ab}	51.7	11 ± 1^{ab}	47.2
4.	25	9.7 ± 0.6^a	45.5	8.7 ± 1.2^a	42	9.3 ± 1.2^a	39.9
5.	PC	21.3 ± 0.6^c	100	20.7 ± 1.2^d	100	23.3 ± 1.2^c	100

PC=positive control, %IR= Resistance Index

S.N.	Measurable zone of inhibition of Essential (mm)		
	Conc.(mg/mL)	*C. albicans* ATCC 10231^T	% RI
6.	50	16.3 ± 1.5^c	74.1
7	25	14.3 ± 0.6^{bc}	65
8.	12.5	12.7 ± 1.5^{ab}	57.7
9	6.25	10 ± 1^a	45.5
10	PC	22 ± 1^d	100

%IR= Resistance Index PC=positive control

The petroleum extract consistently exhibits the largest zones of inhibition across all concentrations, suggesting it extracts compounds with more potent antifungal activity against *C. albicans* compared to methanol and chloroform. For all three extracts, as the concentration increases, the zone of inhibition also tends to increase. This indicates a dose-dependent response, where higher concentrations of the extract lead to greater antifungal activity. The % RI decreases with increasing concentration for all extracts, reinforcing the dose-dependent relationship. The petroleum extract shows the lowest % RI values at each concentration, further supporting its superior antifungal potential in this assay. The petroleum extract of *C. benghalensis* demonstrates the highest antifungal activity against *C. albicans* among the tested extracts, with activity increasing as the concentration of the extract increases. The size of the zone of inhibition is related to the level of antimicrobial activity present in the sample. These findings suggest that the petroleum extract may contain a higher concentration of antifungal compounds or compounds with greater efficacy against *C. albicans* (Leite *et al*., 2015).

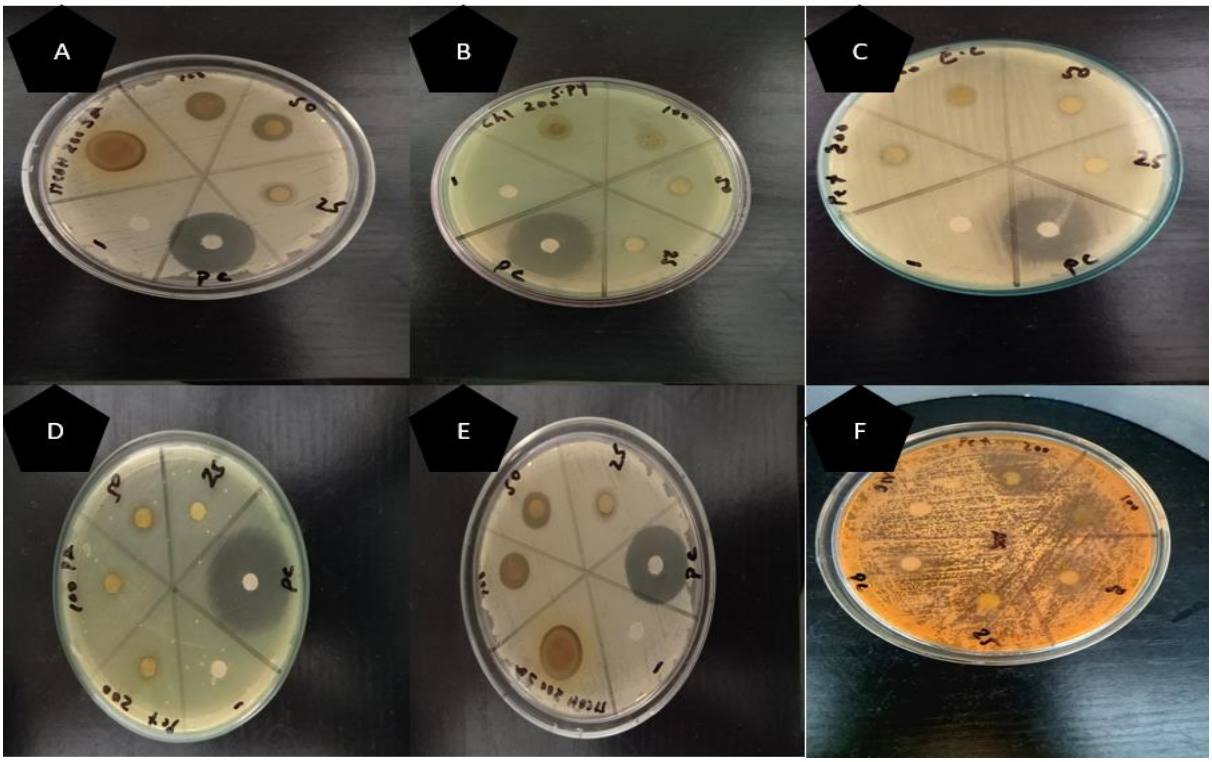


Figure 10: Inhibition zones of *C. benghalensis* extracts against selected human pathogen. A. Methanol extract against *P. aeruginosa,* B. chloroform against *S. pyogens*. C. petroleum against E. Coli D. Petroleum against *P. aeruginosa* E, methanol against *S. aureus* F. petroleum against *C. albican*.

4.5.2 Minimum inhibition concentration, minimum bactericidal and fungicidal concentration (MIC, MBC and MFC)

MIC is defined as the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism after overnight incubation. It serves as a fundamental measure of an antimicrobial's potency against bacteria or fungi. A lower MIC indicates greater effectiveness, as it requires less drug to inhibit microbial growth (Murugappan et al., 2006). MBC is the lowest concentration of an antibacterial agent that results in bacterial death, defined by the inability to recover viable organisms after subculture on antibiotic-free media. The relationship between MIC and MBC can provide insights into whether an agent is bacteriostatic (inhibiting growth) or bactericidal (killing bacteria). When the MBC is close to the MIC, it suggests that the compound has strong bactericidal properties Similar to MBC, MFC refers to the lowest concentration of an antifungal agent that kills a specific fungus. The determination of MFC is particularly important

in treating fungal infections, where both inhibition and killing of the pathogen are critical for effective treatment (Kowalska-Krochmal & Dudek-Wicher, 2021).

Table 6 presents the Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) values for various bacteria and fungi when treated with extracts from methanol, chloroform, petroleum, and essential oils (EO). This discussion analyzes these findings in the context of existing literature to highlight the relative efficacy of different solvents and extracts. *S. pyogenes* (*SPY*) and *E. coli* exhibited MIC values of 50 mg/mL with methanol and 100 mg/mL with chloroform and petroleum extracts. The essential oil showed significantly lower MIC values (12.5 mg/mL), indicating higher potency. *S. aureus* (*SA*) demonstrated similar MIC values across all extracts, while *P. aeruginosa* (PA) showed a notable increase in resistance with higher MICs in chloroform and petroleum extracts. *C. albicans* (*C.A)* had the lowest MIC of 25 mg/mL for methanol extract but a significantly lower value of 6.25 mg/mL for EO, suggesting enhanced antifungal properties.

Table 7: Minimum inhibition concentration, minimum bactericidal and fungicidal concentration (MIC, MBC and MFC) of *C. benghalensis L.* leaf extracts

Bacteria and fungi	Methanol MIC/MBC/MFC (mg/mL)		Chloroform MIC/MBC/MFC (mg/mL)		Petroleum MIC/MBC/MFC (mg/mL)		EO MIC/MBC/MFC (mg/mL)	
SPY	50	100	100	200	100	200	12.5	25
*E.*coli	50	100	100	200	100	200	12.5	25
SA	50	100	50	100	50	100	12.5	25
PA	100	200	50	100	100	200	12.5	25
C.A	25	50	50	100	50	100	6.25	12.5

C.A= Candida albican E. coli= Escherichia coli PA= Pseudomonas aeruginosa SA= Staphylococcus aureus SPY= Streptococcus pyogenes

Comparing these findings with previous studies reveals consistent results regarding the antibacterial properties of *C. benghalensis*. For instance, research has shown that various extracts of this plant possess significant antimicrobial activity against both Gram-positive and Gram-negative bacteria (Adolph, 2016). Specifically, ethanol and methanol extracts have been noted to exhibit strong antibacterial effects against E. coli and *S. aureus*, aligning with the current study's findings.

4.5.3. Antioxidant of crude extracts and EO of *C. benghalensis*

The antioxidant activity in plant extracts mainly comes from compounds like polyphenols, carotenoids, and vitamins. These compounds have various beneficial effects, including reducing inflammation, fighting bacteria and viruses, slowing aging, and preventing cancer (Xu *et al.*, 2017). Plant extracts are a rich source of natural antioxidants, which play a crucial role in maintaining health by combating oxidative stress. The extraction and assessment of these antioxidants are important steps in utilizing them as functional foods, pharmaceuticals, or food

additives. The quantitative antioxidant activity (free radical scavenging activity) of the extracts on the stable radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) was determined by the different methods. In the table 8, the comparision of the antioxidant activities of different extracts from *C. benghalensis L*. leaf (a plant species) with ascorbic acid, a well-known antioxidant presented. The extracts include methanol, chloroform, petroleum ether, and essential oil (E. Oil). A higher %RSA indicates greater antioxidant activity (Loka *et al*., 2024).The antioxidant activity was measured by the %RSA (Percent Radical Scavenging Activity) and absorbance (A) at various concentrations (62.5 to 1000 µg/mL). Ascorbic Acid consistently demonstrated the highest 95%RSA value, confirmed its status as the most potent antioxidant in this study. Methanol and Essential Oil (E. Oil) extracts were also highly effective, both reached 87% RSA at 1000 µg/mL. The Chloroform extract was less effective but still reached 83% RSA at 1000 µg/mL. In contrast, Petroleum Ether was the least effective, with the lowest %RSA values across all concentrations (**Table 7**)**.** The absorbance (A) and radical scavenging activity (%RSA) were shown for each extract at each concentration. A lower absorbance value indicated higher radical scavenging activity. The %RSA represented the percentage of DPPH radicals scavenged by the extract. Ascorbic acid was used as a positive control, shown high radical scavenging activity across all concentrations (Jadid *et al*., 2017).

Table 8: Antioxidant activities of extracts from *C. benghalensis L.*

Cont.	Conc(µg/mL)	Methanol		Chloroform		Petroleum		E. Oil		A. acid	
		A	%RSA	A	%RSA	A	%RSA	A	%RSA	A	%RSA
0.756	62.5	0.23	68	0.29	61	0.421	45	0.25	64	0.092	81
0.756	125	0.18	78	0.26	64	0.391	49	0.18	78	0.079	85
0.756	250	0.14	83	0.20	72	0.346	55	0.14	83	0.072	87
0.756	500	0.13	85	0.17	76	0.304	60	0.13	85	0.056	92
0.756	1000	0.11	87	0.14	83	0.224	78	0.11	87	0.046	95

A=Absorbance, A. acid=Acsorbic acid, Conc.= concentrations, Cont.= control, %RSA= percent of radical scavenging activity

From the current study, results of the Tukey HSD test shown that methanol extract had a significant difference in radical scavenging activity compared to petroleum ether extract, but not with chloroform, essential oil, or ascorbic acid. Chloroform extract did not show significant differences with any of the other extracts. Petroleum ether extract had significant differences with methanol, essential oil, and ascorbic acid. Essential oil differed significantly from petroleum ether but not from methanol, chloroform, or ascorbic acid. Lastly, ascorbic acid showed a significant difference with petroleum ether but not with methanol, chloroform, or essential oil. These findings highlight the distinct antioxidant properties of petroleum ether and ascorbic acid extracts compared to others at a significance level of $p<0.05$. Statistical analysis revealed that methanol and essential oil extracts, as well as ascorbic acid, exhibited significantly higher radical scavenging activity

compared to the petroleum ether extract ($p < 0.05$). This indicated that petroleum ether was less effective antioxidant in this assay than the other tested substances.

A study investigated by Kanta Sahu *et al*., 2013, the anti-oxidant activity of *C. benghalensis* found to a positive correlation between total polyphenols and antioxidant properties of the plant. This plant also provides a great source of dietary antioxidants and possess high antioxidant activities which prevents oxidative damage, slows down the process of aging, reduced the chances of cancer and other cardio-vascular and neurological diseases (Kanta Sahu *et al*., 2013). In the study reported by (Okoko, 2021,) the hydrogen peroxide and hydroxyl radical scavenging capabilities of *C. benghalensis* extracts were investigated. This in vitro assays revealed that the extract effectively scavenged both hydrogen peroxide and hydroxyl radicals. Hydrogen peroxide scavenging activity showed a significant increase between 50 and 100 µg/mL ($p < 0.05$), with no further significant increase between 100 and 200 µg/mL ($p > 0.05$). A significant increase was observed at 500 µg/mL compared to lower concentrations ($p < 0.05$). Hydroxyl radical scavenging displayed a statistically significant concentration-dependent relationship ($p < 0.05$), suggesting a proportional increase in scavenging activity with increasing extract concentration (Okoko, 2021).

5. CONCLUSIONS

This study provided scientific validation for the traditional uses of *C. benghalensis L*. by investigating its phytochemical composition, bioactive compound isolation, antimicrobial and antioxidant activities. The research identified bioactive compound and characterized essential oils, which demonstrated the plant's potential as a source of novel therapeutic agents. The study successfully confirmed the presence of various phytochemicals in *C. benghalensis* leaf extracts, provided a scientific basis for its traditional medicinal uses. It also demonstrated remarkable antimicrobial activity against selected human pathogens, addressing the urgent need for new antimicrobial agents in the face of rising resistance. Furthermore, the investigation revealed the antioxidant activity of the extracts, suggested the potential benefits against oxidative stress and related health issues.

6. RECOMMENDATIONS

To further explore the potential of *C. benghalensis L.*, several avenues of research are suggested. Expanding the isolation process using advanced chromatographic techniques could reveal additional bioactive compounds. A comprehensive phytochemical analysis across various solvents and plant parts would provide a deeper understanding of its chemical composition. Investigating the mechanisms of antimicrobial and antioxidant activities, including synergy with standard antibiotics and broader microbial testing, also recommended. Toxicological and pharmacological studies are essential to establish safety and efficacy profiles. Exploring other biological activities, such as anti-inflammatory and anticancer effects, could uncover new therapeutic applications. Assessing how environmental and seasonal factors affect phytochemical composition is important. Lastly, developing formulations for therapeutic use and evaluating their stability and efficacy in preclinical trials could facilitate drug development. By pursuing these research directions, the medicinal properties of *C. benghalensis L*. can be more fully understood and potentially leveraged for therapeutic purposes.

7. REFEREENCE

Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy & Bioallied Sciences*, *12*(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19

Agency, R. (2002). *Di(2-ethylhexyl)phthalate (DEHP) Public Health Statement*.

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, *46*(1). https://doi.org/10.1186/s42269-022-00770-8

Ahmed, K. B. A., Raman, T., & Veerappan, A. (2016). Future prospects of antibacterial metal nanoparticles as enzyme inhibitor. *Materials Science and Engineering C*, *68*, 939–947. https://doi.org/10.1016/j.msec.2016.06.034

Al-Shahrani, G. S., & Belali, T. M. (2024). Frequency of drug-resistant bacterial isolates among pregnant women with UTI in maternity and children's hospital, Bisha, Saudi Arabia. *Scientific Reports*, *14*(1), 1–8. https://doi.org/10.1038/s41598-024-58275-5

Alade, P. I., & Irobi, O. N. (1993). Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. *Journal of Ethnopharmacology*, *39*(3), 171–174. https://doi.org/10.1016/0378-8741(93)90033-2

Alemu, M., Lulekal, E., Asfaw, Z., Warkineh, B., Debella, A., Abebe, A., Degu, S., & Debebe, E. (2024). Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. *PLoS ONE*, *19*(3 March), 1–15. https://doi.org/10.1371/journal.pone.0300060

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4). https://doi.org/10.3390/plants6040042

Arowora, K. A., Yakubu, O. E., Shaibu, C., Iornenge, T. J., & Ugwuoke, K. C. (2019). *Chemical Composition of Baobab Leaves and Fractionation of its Ethanolic Extract Using Column Chromatography*. *8*(7), 812–821.

Asyikin, Z., Aziz, A., Ahmad, A., Hamidah, S., Setapar, M., & Karakucuk, A. (2018). *Essential Oils : Extraction Techniques , Pharmaceutical And Therapeutic Potential - A Review Essential Oils : Extraction Techniques , Pharmaceutical And Therapeutic Potential - A Review*. *July*. https://doi.org/10.2174/1389200219666180723144850

Aziz, Z. A. A., Ahmad, A., Setapar, S. H. M., Karakucuk, A., Azim, M. M., Lokhat, D., Rafatullah, M., Ganash, M., Kamal, M. A., & Ashraf, G. M. (2018). Essential Oils: Extraction Techniques, Pharmaceutical And Therapeutic Potential - A Review. *Current Drug Metabolism*, *19*(13), 1100–1110. https://doi.org/10.2174/1389200219666180723144850

Bamola, N., Verma, P., & Negi, C. (2018). A Review on Some Traditional Medicinal Plants. *International Journal of Life-Sciences Scientific Research*, *4*(1). https://doi.org/10.21276/ijlssr.2018.4.1.7

Bitwell, C., Indra, S. Sen, Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, *19*, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585

Cavichi, L. V., Liberal, Â., Dias, M. I., Mandim, F., Pinela, J., Kostić, M., Soković, M., Kalschne, D. L., Fernandes, Â., Canan, C., Barros, L., & Amaral, J. S. (2023). Chemical Composition and Biological Activity of Commelina erecta: An Edible Wild Plant Consumed in Brazil. *Foods*, *12*(1), 1–15. https://doi.org/10.3390/foods12010192

Chioma, V., & Omoregie, H. (2010). Pharmacognostic and Phytochemical Analysis of Commelina benghalensis L. *Ethnobotanical Leaflets*, *14*(Burkill 2000), 610–625.

Choi, S. J., Kim, J. K., Kim, H. K., Harris, K., Kim, C. J., Park, G. G., Park, C. S., & Shin, D. H. (2013). 2,4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12

cells and in mice. *Journal of Medicinal Food*, *16*(11), 977–983. https://doi.org/10.1089/jmf.2012.2739

Coleman, W. M., Dube, M. F., Ashraf-Khorassani, M., & Taylor, L. T. (2007). Isomeric enhancement of davanone from natural davana oil aided by supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry*, *55*(8), 3037–3043. https://doi.org/10.1021/jf062652y

Cross, S., & Science, P. (2019). *Terpenoids and phytosteroids isolated from Commelina benghalensis Linn . with antioxidant Abstract :* 1–7. https://doi.org/10.1515/jbcpp-2018-0218

Cuéllar Cuéllar, A., & Okori, O. D. (2010). Preliminary Phytochemical and Antimicrobial Evaluation of the Fresh and Dried Whole Plant Extracts from Commelina benghalensis. *Revista Colombiana de Ciência Animal*, *2*(1), 104–116.

Das, S., Pal, D., Singharoy, S., Lohar, P., Hazra, S., & Ghosh, D. (2024). *International Journal of Pharmaceutical Sciences and Drug Research Salutary Effect of Commelina benghalensis (Linn .) Aerial Parts on Streptozotocin Induced Diabetes Linked Complications in Rat : Effective Extract Selection Study*. *16*(6), 1013–1022. https://doi.org/10.25004/IJPSDR.2024.160612

Den Braver-Sewradj, S. P., Piersma, A., & Hessel, E. V. S. (2020). An update on the hazard of and exposure to diethyl hexyl phthalate (DEHP) alternatives used in medical devices. *Critical Reviews in Toxicology*, *50*(8), 650–672. https://doi.org/10.1080/10408444.2020.1816896

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*, *15*(January), 51–62. https://doi.org/10.2147/JEP.S379805

El-bashiti, T. A., Elkhair, E. A., & Draz, W. S. A. (2017). The Antibacterial and Synergistic

Potential of some Palestinian Plant Extracts Against Multidrug Resistant Staphylococcus aureus. *Journal of Medicinal Plants Studies*, *5*(2), 54–65.

Esmaeili, H., Karami, A., & Maggi, F. (2018). Essential oil composition, total phenolic and flavonoids contents, and antioxidant activity of Oliveria decumbens Vent. (Apiaceae) at different phenological stages. *Journal of Cleaner Production*, *198*, 91–95. https://doi.org/10.1016/j.jclepro.2018.07.029

Fibrich, B., & Lall, N. (2020). *Commelina benghalensis*. *D*, 77–85. https://doi.org/10.1016/B978-0-12-816814-1.00011-9

File, T., & Dinka, H. (2020). A preliminary study on urban malaria during the minor transmission season: The case of Adama City, Oromia, Ethiopia. *Parasite Epidemiology and Control*, *11*, e00175. https://doi.org/10.1016/j.parepi.2020.e00175

Gebrehiwot, H., Ensermu, U., Dekebo, A., Endale, M., & Nefo Duke, T. (2024). In Vitro Antibacterial and Antioxidant Activities, Pharmacokinetics, and In Silico Molecular Docking Study of Phytochemicals from the Roots of Ziziphus spina-christi . *Biochemistry Research International*, *2024*(1). https://doi.org/10.1155/2024/7551813

Ghosh, P., Dutta, A., Biswas, M., Biswas, S., Hazra, L., Kumar Nag, S., Sil, S., Chatterjee, S., & Sirshendu Chatterjee, C. (2019). Phytomorphological, chemical and pharmacological discussions about Commelina benghalensis Linn. (Commelinaceae): A review. *~ 12 ~ The Pharma Innovation Journal*, *8*(6), 12–18. www.thepharmajournal.com

Gutierrez-Rubio, S., Shamzhy, M., Cejka, J., Serrano, D., Coronado, J., & Moreno, I. (2021). Synthesis of cyclohexylphenol via phenol hydroalkylation using Co2P/zeolite catalysts. *Catalysis Today*, *390–391*. https://doi.org/10.1016/j.cattod.2021.11.039

Harini, V., & Jayanthi, K. (2022). *Phytochemical analysis and identification of active compounds using GC-MS analysis of Commelina benghalensis*. *4*(9), 582–592. https://doi.org/10.35629/5252-0409582592

Hemalatha, M., Thirumalai, T., Saranya, R., Elumalai, K., & David, E. (2013). A review on

antimicrobial efficacy of some traditional medicinal plants in Tamilnadu. *Journal of Acute Disease*, *2*(2), 99–105. https://doi.org/10.1016/S2221-6189(13)60107-9

Identity, C. (2013). *CHAPTER 4 . CHEMICAL AND PHYSICAL INFORMATION Di (2-ethylhexyl) phthalate , also known as DEHP , is an organic ester containing an eight-carbon alcohol moiety widely used as a plasticizer in polymers . DEHP is widely used for a variety of standard produ*. *117*, 355–356.

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., & Wikanta, W. (2017). Antioxidant activities of different solvent extracts of Piper retrofractum Vahl. using DPPH assay. *AIP Conference Proceedings*, *1854*(December). https://doi.org/10.1063/1.4985410

Kalia, A., Kaur, M., Shami, A., Jawandha, S. K., Alghuthaymi, M. A., Thakur, A., & Abd-Elsalam, K. A. (2021). Nettle-leaf extract derived zno/cuo nanoparticle-biopolymer-based antioxidant and antimicrobial nanocomposite packaging films and their impact on extending the post-harvest shelf life of guava fruit. *Biomolecules*, *11*(2), 1–24. https://doi.org/10.3390/biom11020224

Kansagara, P. A., & Pandya, D. J. (2023). Best From Waste: Bioactivity-Guided Formulation Development From A Common Weed - Commelina benghalensis. *International Journal of Pharmaceutical Sciences and Drug Research*, *15*(3), 331–341. https://doi.org/10.25004/ijpsdr.2023.150314

Kanta Sahu, R., Kar, M., & Routray, R. (2013). DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha, India. *Journal of Medicinal Plants Studies Year*, *1*(1), 2013. www.plantsjournal.comwww.plantsjournal.com

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS One*, *16*(3), e0249253. https://doi.org/10.1371/journal.pone.0249253

Khanpara, P. (2019). A Complete Review on Medicinally Active Herbal Weed: Commelina

benghalensis L. (Commelinaceae). . *Journal of Pharmaceutical Sciences and Research. 2019; 11(4): 1165-71 [ISSN: 0975-1459; h-Index: 18; SNIP: 0.38, Scopus-Indexed]*., *June*.

Khanpara, P., & Pandya, D. D. (2023). Best From Waste: Bioactivity-Guided Formulation Development From A Common Weed - Commelina benghalensis. In *International Journal of Pharmaceutical Sciences and Drug Research* (Vol. 15). https://doi.org/10.25004/IJPSDR.2023.150314

Khatun, A., Rahman, M., Rahman, M. S., Hossain, M. K., & Rashid, M. A. (2019). Terpenoids and phytosteroids isolated from Commelina benghalensis Linn. with antioxidant activity. *Journal of Basic and Clinical Physiology and Pharmacology*, *31*(1). https://doi.org/10.1515/jbcpp-2018-0218

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, *10*(2), 1–21. https://doi.org/10.3390/pathogens10020165

Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, V., & Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules*, *28*(2), 1–41. https://doi.org/10.3390/molecules28020887

Leite, M. C. A., De Brito Bezerra, A. P., De Sousa, J. P., & De Oliveira Lima, E. (2015). Investigating the antifungal activity and mechanism(s) of geraniol against Candida albicans strains. *Medical Mycology*, *53*(3), 275–284. https://doi.org/10.1093/mmy/myu078

Liu, W., Hsu, Y. Y., Tang, J. Y., Cheng, Y. Bin, Chuang, Y. T., Jeng, J. H., Yen, C. H., & Chang, H. W. (2022). Methanol Extract of Commelina Plant Inhibits Oral Cancer Cell Proliferation. *Antioxidants*, *11*(9). https://doi.org/10.3390/antiox11091813

Loka, S., Rakesh, M. M., Anandan, J., & Shanmugam, R. (2024). Free Radical Scavenging Activity of Commelina Benghalensis Mediated Calcium Oxide Nanoparticles. *Nanotechnology Perceptions*, *20*(S7), 467–476. https://doi.org/10.62441/nano-ntp.v20iS7.41

Maharjan, B. L., Mainali, S., & Baral, B. (2011). Phytochemical Screening and Antimicrobial. *Scientific World*, *9*(9), 90–92.

Malarvizhi, D., Karthikeyan, A. V. P., Sudan, I., & Satheeshkumar, R. (2019). Phytochemical analysis of Commelina diffusa Burm. F. through GC-MS method. *Journal of Pharmacognosy and Phytochemistry*, *8*(1), 376–379.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *Journal of Tropical Medicine, 2019*, 1895340. https://doi.org/10.1155/2019/1895340

Muniyandi, J. M., Jothi Muniyandi, M., & Professor, A. (2018). Preliminary Studies of Phytochemical Investigation on Coastal Medicinal Plants of Boloor, Mangalore. *Indo Am. J. P. Sci*, *05*(02), 5. http://doi.org/10.5281/zenodo.1196309

Murugappan, A., Sudarsan, J. S., & Manoharan, A. (2006). Effects of using Lignite mine drainage for irrigation on soils - A case study of perumal tank command area in Tamilnadu State. *Journal of Industrial Pollution Control*, *22*(1), 149–160.

Nazim, N. B., & Khatun, A. (2024). *Traditional Uses , Phytochemistry and Pharmacology of Ipomoea Carnea : an Updated Systematic*. *10*(May), 53–59.

Okoko, T. (2021). Assessment of the Inherent in-vitro Antioxidant Potential of Commelina benghalensis Leaf Extract. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, *6*(4), 25–32. https://doi.org/10.9734/ajbgmb/2020/v6i430159

Pandey, A., & Tripathi, S. (2014). *Concept of standardization , extraction and pre phytochemical screening strategies for herbal drug*. *2*(5), 115–119.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health*, *109*(7), 309–318. https://doi.org/10.1179/2047773215Y.0000000030

Purushothaman, S., & Sumithra, D. (2017). phytochemical profiling of ethanolic leaves ,extract

of Commelina benghalensis L. *World Journal of Pharmaceutical Research*, *6*(2), 1101–1107. https://doi.org/10.20959/wjpr20172-7817

Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, *100*(1–2), 80–84. https://doi.org/10.1016/j.jep.2005.04.025

Rodr, C., Alonso-calleja, C., Garc, C., Carballo, J., & Capita, R. (2022). Bactericidal Concentration (MBC) for Twelve Antimicrobials. *Biology*, *11*(Mic), 46.

Rowdhwal, S. S. S., & Chen, J. (2018). Toxic Effects of Di-2-ethylhexyl Phthalate: An Overview. *BioMed Research International*, *2018*(Figure 1). https://doi.org/10.1155/2018/1750368

Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. A. (2023). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare (Basel, Switzerland)*, *11*(13). https://doi.org/10.3390/healthcare11131946

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, *8*(1), 1–10.

Silva, F., Lourenço, O., Queiroz, J. A., & Domingues, F. C. (2011). Bacteriostatic versus bactericidal activity of ciprofloxacin in Escherichia coli assessed by flow cytometry using a novel far-red dye. *Journal of Antibiotics*, *64*(4), 321–325. https://doi.org/10.1038/ja.2011.5

Sinha, U. (2019). Analysis of phytoconstituents and antimicrobial properties of leaf extract of Commenila benghalensis L, against selected microbes. *International Journal of Scientific and Research Publications (IJSRP)*, *9*(4), p8841. https://doi.org/10.29322/ijsrp.9.04.2019.p8841

Tadesse, S., & Ganesan, K. (2016a). Preliminary phytochemical screening of different solvent extracts of leaves and stems of Commelina Benghalensis L (Family: Commelinaceae) Insect proteins: Therapeutic effects on human body View project Cellular Signaling Metabolic

Diseases View project. *Article in Research Journal of Pharmaceutical Biological and Chemical Sciences*, *January*. https://www.researchgate.net/publication/329208557

Tadesse, S., & Ganesan, K. (2016b). Preliminary phytochemical screening of different solvent extracts of leaves and stems of Commelina Benghalensis L (Family: Commelinaceae) Insect proteins: Therapeutic effects on human body View project Cellular Signaling Metabolic Diseases View project. *Article in Research Journal of Pharmaceutical Biological and Chemical Sciences*, *6*(1), 103–107. https://www.researchgate.net/publication/329208557

Tâm, T., Và, N. C. Ứ U., Giao, C. Ề N., Ngh, C., & Chu, Ả N B Ụ I. (2016). *济無No Title No Title No Title*. *01*(10), 1–23. https://doi.org/10.13040/IJPSR.0975-8232.IJP.5(10).637-45

Technique, I., Bioactive, O. F., & From, M. (2013). *International Journal of Current Tropical Medicine and Health Research Isolation Technique of Bioactive Molecules From Plants and Their Applications*. *1*, 1–4.

Tesfaye, S., Belete, A., Engidawork, E., Gedif, T., & Asres, K. (2020). Ethnobotanical Study of Medicinal Plants Used by Traditional Healers to Treat Cancer-Like Symptoms in Eleven Districts, Ethiopia. *Evidence-Based Complementary and Alternative Medicine*, *2020*. https://doi.org/10.1155/2020/7683450

Usure, R. E., Kebebe, D., Mekasha, Y. T., Hasen, G., Chura Waritu, N., Dubale, S., & Suleman, S. (2024). Traditional herbal medicine regulatory implementation in Ethiopia: a qualitative study. *Frontiers in Pharmacology*, *15*(April), 1–14. https://doi.org/10.3389/fphar.2024.1392330

Valgas, C., De Souza, S. M., Smânia, E. F. A., & Smânia, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 369–380. https://doi.org/10.1590/S1517-83822007000200034

Wolditsadik, M. (2018). Traditional Medicinal Plants in Ethiopia. *International Journal of Biology, Physics & Matematics*, *1*(1), 80–87.

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J., & Li, H.-B. (2017).

Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International Journal of Molecular Sciences*, *18*(1). https://doi.org/10.3390/ijms18010096

8. APPENDIX

Table A1: Significance Crude Extracts and Essential Oil against of E. coli ATTC 25922T

Multiple Comparisons

Dependent Variable: Inhibition zone (mm)

Tukey HSD

(I) Plant_Extracts against E. coli	(J) Plant_Extracts against E. coli	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence I Lower Bound	Up
MeoH against E.coli	Chlo against E.coli	1.667	.721	.156	-.37	
	Petr against E.coli	2.417*	.721	.012	.38	
	ciprooxacin against E.coli	-13.833*	.721	.000	-15.87	
	EO against E.coli	-.917	.721	.709	-2.95	
Chlo against E.coli	MeoH against E.coli	-1.667	.721	.156	-3.70	
	Petr against E.coli	.750	.721	.835	-1.28	
	ciprooxacin against E.coli	-15.500*	.721	.000	-17.53	
	EO against E.coli	-2.583*	.721	.006	-4.62	
Petr against E.coli	MeoH against E.coli	-2.417*	.721	.012	-4.45	
	Chlo against E.coli	-.750	.721	.835	-2.78	
	ciprooxacin against E.coli	-16.250*	.721	.000	-18.28	
	EO against E.coli	-3.333*	.721	.000	-5.37	
ciprooxacin against E.coli	MeoH against E.coli	13.833*	.721	.000	11.80	
	Chlo against E.coli	15.500*	.721	.000	13.47	
	Petr against E.coli	16.250*	.721	.000	14.22	
	EO against E.coli	12.917*	.721	.000	10.88	
EO against E.coli	MeoH against E.coli	.917	.721	.709	-1.12	
	Chlo against E.coli	2.583*	.721	.006	.55	
	Petr against E.coli	3.333*	.721	.000	1.30	
	ciprooxacin against E.coli	-12.917*	.721	.000	-14.95	

*. The mean difference is significant at the 0.05 level.

Table A2: Significance Crude Extracts and Essential Oil against *S. aures* ATCC 25923^{T}

Multiple Comparisons

Dependent Variable: Inhibition_zone (mm)

Tukey HSD

(I) Plant_Extracts against SA	(J) Plant_Extracts against SA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
MeoH against SA	Chlo against SA	-.333	.973	.997	-3.08	2.41
	Petr against SA	1.167	.973	.752	-1.58	3.91
	PC (ciprooxacin) against SA	-12.833*	.973	.000	-15.58	-10.09
	EO against SA	-1.500	.973	.541	-4.24	1.24
Chlo against SA	MeoH against SA	.333	.973	.997	-2.41	3.08
	Petr against SA	1.500	.973	.541	-1.24	4.24
	PC (ciprooxacin) against SA	-12.500*	.973	.000	-15.24	-9.76
	EO against SA	-1.167	.973	.752	-3.91	1.58
Petr against SA	MeoH against SA	-1.167	.973	.752	-3.91	1.58
	Chlo against SA	-1.500	.973	.541	-4.24	1.24
	PC (ciprooxacin) against SA	-14.000*	.973	.000	-16.74	-11.26
	EO against SA	-2.667	.973	.061	-5.41	.08
PC (ciprooxacin) against SA	MeoH against SA	12.833*	.973	.000	10.09	15.58
	Chlo against SA	12.500*	.973	.000	9.76	15.24
	Petr against SA	14.000*	.973	.000	11.26	16.74
	EO against SA	11.333*	.973	.000	8.59	14.08
EO against SA	MeoH against SA	1.500	.973	.541	-1.24	4.24
	Chlo against SA	1.167	.973	.752	-1.58	3.91
	Petr against SA	2.667	.973	.061	-.08	5.41
	PC (ciprooxacin) against SA	-11.333*	.973	.000	-14.08	-8.59

*. The mean difference is significant at the 0.05 level.

Table A3: Significance Crude Extracts and Essential Oil against *P. aeuruginosa* ATCC 27853^{T}

Multiple Comparisons

Dependent Variable: Inhibition zone (mm)

Tukey HSD

(I) Plant_Extracts against PA	(J) Plant_Extracts against PA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
MeoH against PA	Chlo against PA	.333	.772	.993	-1.84	2.51
	Petr against PA	2.167	.772	.052	-.01	4.34
	ciprooxacin against PA	-16.750*	.772	.000	-18.93	-14.57
	EO against PA	-2.417*	.772	.022	-4.59	-.24
Chlo against PA	MeoH against PA	-.333	.772	.993	-2.51	1.84
	Petr against PA	1.833	.772	.137	-.34	4.01
	ciprooxacin against PA	-17.083*	.772	.000	-19.26	-14.91
	EO against PA	-2.750*	.772	.007	-4.93	-.57
Petr against PA	MeoH against PA	-2.167	.772	.052	-4.34	.01
	Chlo against PA	-1.833	.772	.137	-4.01	.34
	ciprooxacin against PA	-18.917*	.772	.000	-21.09	-16.74
	EO against PA	-4.583*	.772	.000	-6.76	-2.41
ciprooxacin against PA	MeoH against PA	16.750*	.772	.000	14.57	18.93
	Chlo against PA	17.083*	.772	.000	14.91	19.26
	Petr against PA	18.917*	.772	.000	16.74	21.09
	EO against PA	14.333*	.772	.000	12.16	16.51
EO against PA	MeoH against PA	2.417*	.772	.022	.24	4.59
	Chlo against PA	2.750*	.772	.007	.57	4.93
	Petr against PA	4.583*	.772	.000	2.41	6.76
	ciprooxacin against PA	-14.333*	.772	.000	-16.51	-12.16

*. The mean difference is significant at the 0.05 level.

Table A4: Significance Crude Extracts and Essential Oil against *S. pyogene* ATCC 12204^T

Multiple Comparisons

Dependent Variable: Inhibition zone (mm)

Tukey HSD

(I) Plant_Extracts against SPY	(J) Plant_Extracts against SPY	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
MeoH against SPY	Chlo against SPY	1.917	.833	.160	-.43	4.27
	Petr against SPY	3.083*	.833	.004	.73	5.43
	ciprooxacin against SPY	-14.167*	.833	.000	-16.52	-11.82
	EO against SPY	-1.167	.833	.630	-3.52	1.18
Chlo against SPY	MeoH against SPY	-1.917	.833	.160	-4.27	.43
	Petr against SPY	1.167	.833	.630	-1.18	3.52
	ciprooxacin against SPY	-16.083*	.833	.000	-18.43	-13.73
	EO against SPY	-3.083*	.833	.004	-5.43	-.73
Petr against SPY	MeoH against SPY	-3.083*	.833	.004	-5.43	-.73
	Chlo against SPY	-1.167	.833	.630	-3.52	1.18
	ciprooxacin against SPY	-17.250*	.833	.000	-19.60	-14.90
	EO against SPY	-4.250*	.833	.000	-6.60	-1.90
ciprooxacin against SPY	MeoH against SPY	14.167*	.833	.000	11.82	16.52
	Chlo against SPY	16.083*	.833	.000	13.73	18.43
	Petr against SPY	17.250*	.833	.000	14.90	19.60
	EO against SPY	13.000*	.833	.000	10.65	15.35
EO against SPY	MeoH against SPY	1.167	.833	.630	-1.18	3.52
	Chlo against SPY	3.083*	.833	.004	.73	5.43
	Petr against SPY	4.250*	.833	.000	1.90	6.60
	ciprooxacin against SPY	-13.000*	.833	.000	-15.35	-10.65

*. The mean difference is significant at the 0.05 level.

Table A5: Significance Crude Extracts and Essential Oil against *C. albicans* ATCC 10231^T

Multiple Comparisons

Dependent Variable: Inhibition zone (mm)

Tukey HSD

(I) Plant_Extracts against C.A	(J) Plant_Extracts against C.A	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
MeoH against C.A	Chlo against C.A	.500	1.048	.989	-2.45	3.45
	Petr against C.A	-.667	1.048	.968	-3.62	2.29
	Ketoconazole against C.A	-6.917*	1.048	.000	-9.87	-3.96
	EO against C.A	-1.500	1.048	.610	-4.45	1.45
Chlo against C.A	MeoH against C.A	-.500	1.048	.989	-3.45	2.45
	Petr against C.A	-1.167	1.048	.799	-4.12	1.79
	Ketoconazole against C.A	-7.417*	1.048	.000	-10.37	-4.46
	EO against C.A	-2.000	1.048	.325	-4.95	.95
Petr against C.A	MeoH against C.A	.667	1.048	.968	-2.29	3.62
	Chlo against C.A	1.167	1.048	.799	-1.79	4.12
	Ketoconazole against C.A	-6.250*	1.048	.000	-9.20	-3.30
	EO against C.A	-.833	1.048	.931	-3.79	2.12
Ketoconazole against C.A	MeoH against C.A	6.917*	1.048	.000	3.96	9.87
	Chlo against C.A	7.417*	1.048	.000	4.46	10.37
	Petr against C.A	6.250*	1.048	.000	3.30	9.20
	EO against C.A	5.417*	1.048	.000	2.46	8.37
EO against C.A	MeoH against C.A	1.500	1.048	.610	-1.45	4.45
	Chlo against C.A	2.000	1.048	.325	-.95	4.95
	Petr against C.A	.833	1.048	.931	-2.12	3.79
	Ketoconazole against C.A	-5.417*	1.048	.000	-8.37	-2.46

*. The mean difference is significant at the 0.05 level.

Table A6: Significance Crude Extracts and Essential Oil against *C. albicans* ATCC 10231^T

Multiple Comparisons

Dependent Variable: Radical Scavenging Assay

Tukey HSD

(I) Plant extracts	(J) Plant extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
methanol	Chloroform	9.000	5.790	.541	-8.32	26.32
	Petroleum ether	22.800*	5.790	.006	5.48	40.12
	Essential Oil	.800	5.790	1.000	-16.52	18.12
	Acsorbic acid	-7.800	5.790	.666	-25.12	9.52
Chloroform	methanol	-9.000	5.790	.541	-26.32	8.32
	Petroleum ether	13.800	5.790	.161	-3.52	31.12
	Essential Oil	-8.200	5.790	.625	-25.52	9.12
	Acsorbic acid	-16.800	5.790	.060	-34.12	.52
Petroleum ether	methanol	-22.800*	5.790	.006	-40.12	-5.48
	Chloroform	-13.800	5.790	.161	-31.12	3.52
	Essential Oil	-22.000*	5.790	.009	-39.32	-4.68
	Acsorbic acid	-30.600*	5.790	.000	-47.92	-13.28
Essential Oil	methanol	-.800	5.790	1.000	-18.12	16.52
	Chloroform	8.200	5.790	.625	-9.12	25.52
	Petroleum ether	22.000*	5.790	.009	4.68	39.32
	Acsorbic acid	-8.600	5.790	.583	-25.92	8.72
Acsorbic acid	methanol	7.800	5.790	.666	-9.52	25.12
	Chloroform	16.800	5.790	.060	-.52	34.12
	Petroleum ether	30.600*	5.790	.000	13.28	47.92
	Essential Oil	8.600	5.790	.583	-8.72	25.92

*. The mean difference is significant at the 0.05 level.

Table A$_7$: Phytochemical Screening of *Commelina benghalensis L.*

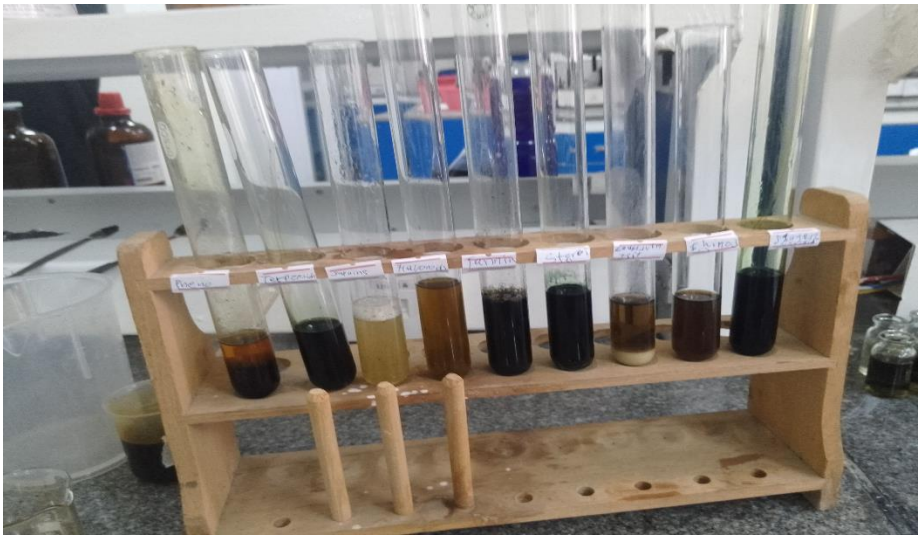


Table A$_8$*:* Machine of GC-MS analysis

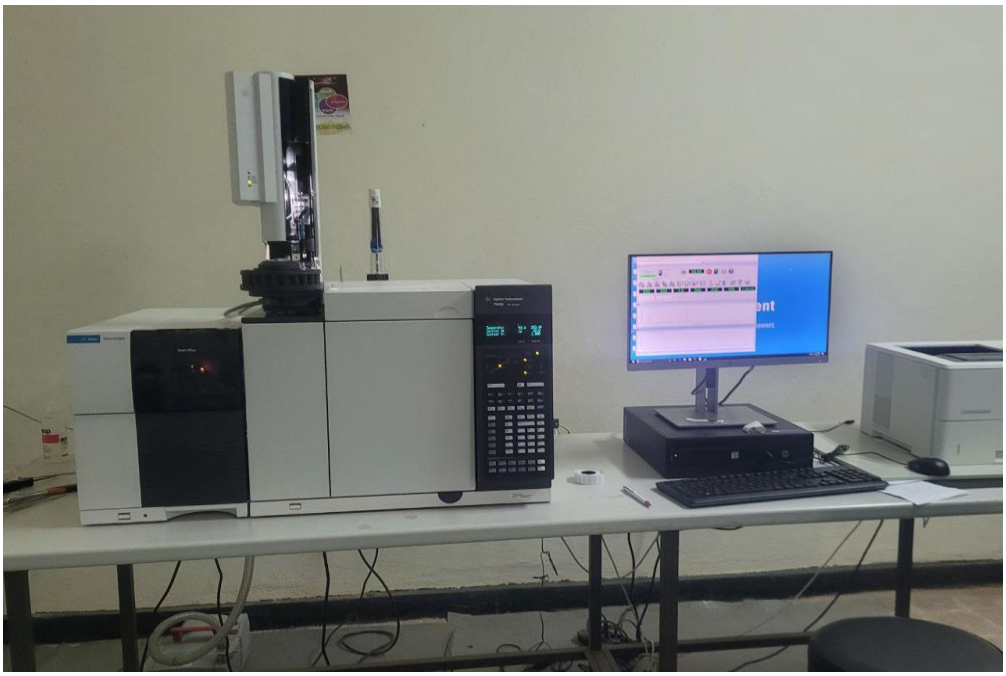


Table A$_{9}$: TLC test for crudes before column chromatograpy

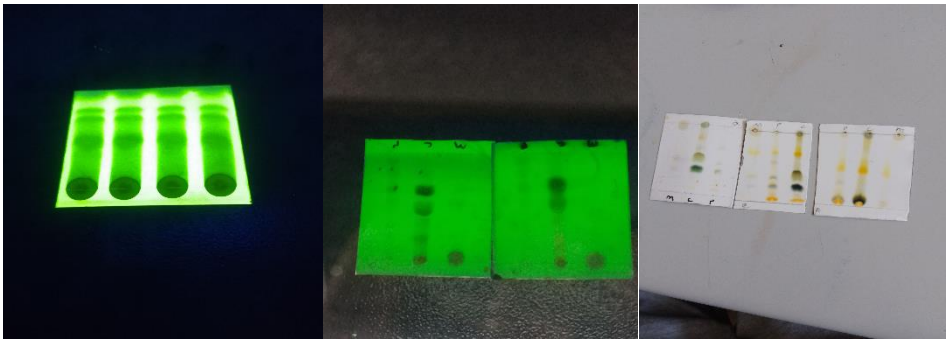


Table A$_{9}$: Separatory funnel to remove chlorophyll

Table A$_{10}$: TLC test after column chromatograpy

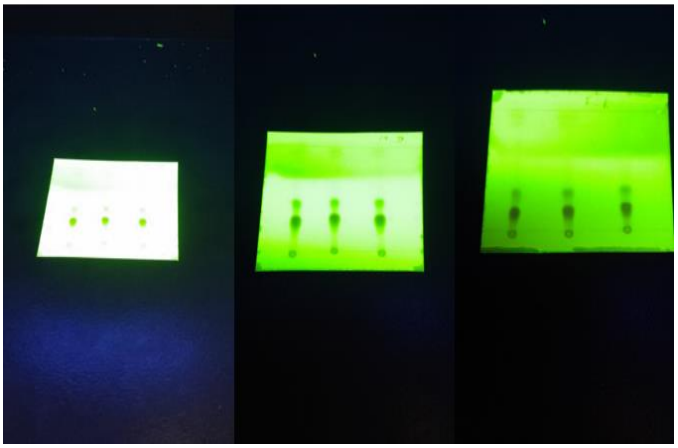


Table A11: Minimum inhibition concentration test

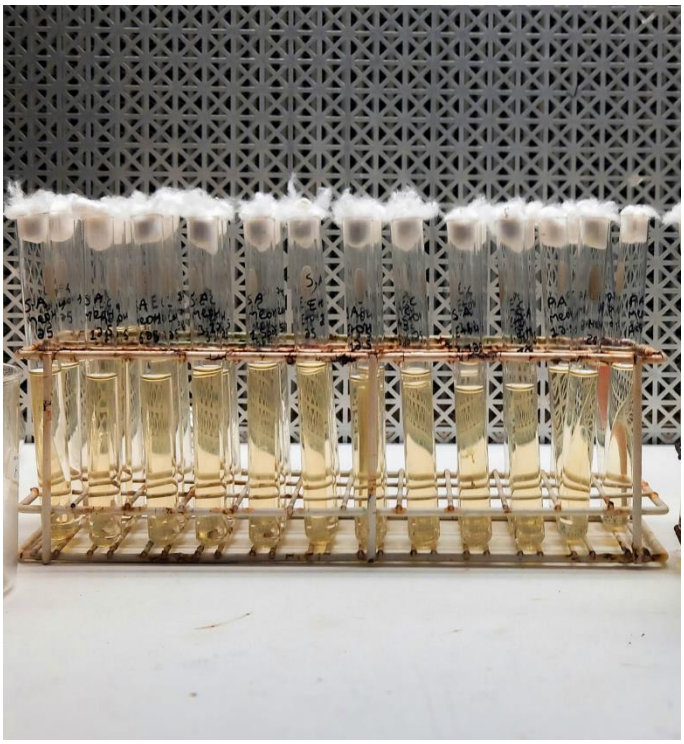


Table A12: Separation of essential oil from water